(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 870 839 A1

(12) EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

AC

(43) Date of publication:
14.10.1998 Bulletin 1998/42

(21) Application number: **96937515.3**

(22) Date of filing: **07.11.1996**

(51) Int. Cl.[6]: **C12N 15/86**, C12N 15/12, C12N 5/10

(86) International application number:
PCT/JP96/03254

(87) International publication number:
WO 97/18318 (22.05.1997 Gazette 1997/22)

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **13.11.1995 JP 294382/95**
08.03.1996 JP 51847/96

(83) **Declaration under Rule 28(4) EPC (expert solution)**

(71) Applicant: **TAKARA SHUZO CO. LTD.**
Kyoto-shi, Kyoto 612 (JP)

(72) Inventors:
- **ASADA, Kiyozo**
  Koka-gun, Shiga 520-33 (JP)
- **UEMORI, Takashi**
  Otsu-shi, Shiga 520-21 (JP)
- **UENO, Takashi**
  Kusatsu-shi, Shiga 525 (JP)
- **KOYAMA, Nobuto**
  Uji-shi, Kyoto 611 (JP)
- **HASHINO, Kimikazu**
  Takatsuki-shi, Osaka 569 (JP)
- **KATO, Ikunoshiu**
  Uji-shi, Kyoto 611 (JP)

(74) Representative:
Vossius, Volker, Dr. et al
Dr. Volker Vossius,
Patentanwaltskanzlei - Rechtsanwaltskanzlei,
Holbeinstrasse 5
81679 München (DE)

(54) METHOD FOR GENE INTRODUCTION INTO TARGET CELLS BY RETROVIRUS

(57) A method for increasing the efficiency of gene transfer into target cells with a *retrovirus* is disclosed. In the method, the target cells are infected with the retrovirus in the presence of either a mixture of an effective amount of a functional material having retrovirus binding domain and an effective amount of another functional material having target cell binding domain, or an effective amount of a functional material having these binding domains on the same molecule. The functional materials may be used without immobilization or with immobilized on beads. The method is useful, for example, gene therapy.

EP 0 870 839 A1

transfer method.

SUMMARY OF THE INVENTION

The present inventors have found that retrovirus infection by a functional material, typically, fibronectin or its fragment, can be promoted, even when a region having a retrovirus binding domain and a region having a cell binding domain are not present on the same molecule. That is, the present inventors have found that the efficiency of gene transfer into target cells by retroviruses can be improved by using an effective amount of a functional material containing a retrovirus binding domain admixed with a functional material having a target cell binding domain.

In addition, the present inventors have also found that retrovirus infection enhancing activity by a functional material can be observed even when the functional material is not immobilized on a surface of a plate. The present inventors have further found that the efficiency of gene transfer into target cells can be improved by contacting retroviruses with the target cells in the presence of a functional material immobilized on beads.

In addition, the present inventors have further found a retrovirus binding substance which does not contain a heparin binding domain derived from fibronectin and also found that the material and derivatives thereof are useful for gene transfer into target cells with retroviruses. Moreover, the present inventors have succeeded in creation of functional materials useful for gene transfer into target cells with retroviruses. Thus, the present invention has been completed.

Then, the first aspect of the present invention relates to a method for increasing the efficiency of gene transfer into target cells with retroviruses. The method is directed to transduction of target cells with a retrovirus and is characterized by infecting the target cells with the retrovirus in the presence of a mixture of an effective amount of a functional material having retrovirus binding domain, and an effective amount of another functional material having target cell binding domain to permit transfer of the gene into the target cells.

The functional material having retrovirus binding domain used in the first aspect of the present invention is not specifically limited and, for example, it is a functional material selected from the group consisting of the Heparin-II binding domain of fibronectin, a fibroblast growth factor, a collagen, a polylysine and functional equivalents thereof. The functional material having target cell binding domain may be a substance containing a ligand which can bind to target cells. As the ligand, there are cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites of target cells and the like. Examples of adhesion proteins include polypeptides of a cell binding domain of fibronectin. As the cell binding domain of fibronectin, there are polypeptides of binding domain to VLA-5 and/or VLA-4. Further, other examples of ligand include erythropoietin.

The functional material to be used in the first aspect of the present invention may be used without immobilization or may be immobilized and, when they are immobilized on beads, they can be used conveniently. In addition, when a ligand specific for target cells is selected as the functional material having target cell binding domain, the first aspect of the present invention permits convenient transduction of intended target cells.

As described above, in the conventional methods as disclosed in WO 95/26200 and Nature Medicine, it is consider to be an essential mechanism for improving the gene transfer efficiency into target cells with a retrovirus to co-localize the retrovirus and the target cells on a functional material having both retrovirus binding domain and target cell binding domain on the same molecule. However, according to the present invention, the efficiency of gene transfer into target cells can be improved by carrying out gene transfer into the target cells with a retrovirus in the presence of a mixture of an effective amount of a functional material having retrovirus binding domain and an effective amount of another functional material having target cell binding domain.

The second aspect of the present invention relates to a culture medium for target cells to be used for gene transfer into the target cells with retroviruses which comprises a mixture of an effective amount of a functional material having retrovirus binding domain, and an effective amount of another functional material having target cell binding domain.

By using the culture medium of the second aspect of the present invention, the first aspect of the present invention can be carried out conveniently.

The third aspect of the present invention relates to a localization method of retroviruses and the method is characterized by incubating a culture medium containing a retrovirus contacted with a mixture of an effective amount of a functional material having retrovirus binding domain, and an effective amount of another functional material having target cell binding domain.

The fourth aspect of the present invention relates to a kit to be used for carrying out retrovirus-mediated gene transfer into target cells and the kit comprises:

(a) an effective amount of a functional material having retrovirus binding domain and/or an effective amount of another functional material having target cell binding domain;
(b) an artificial substrate for incubating target cells and a retrovirus; and
(c) a target cell growth factor for pre-stimulating the target cells.

immobilized, though immobilization is preferred in case that target cells are adherent cells.

The sixth aspect of the present invention relates to a culture medium for target cells to be used for gene transfer into the target cells with a retrovirus which comprises an effective amount of a functional material which has a target cell binding domain as well as a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

The seventh aspect of the present invention relates to a localization method of a retrovirus which comprises incubating a culture medium containing the retrovirus contacted with a effective amount of a functional material containing a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine. These functional materials can be efficiently used in localization of a retrovirus for improvement of gene transfer into target cells with the retrovirus.

Moreover, the localization method of a retrovirus of the present invention include incubation of the retrovirus contacted with an effective amount of a functional material comprising a target cell bind domain as well as a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

The eighth aspect of the present invention is a kit to be used for carrying out retrovirus-mediated gene transfer into target cells and the kit comprises:

(a) an effective amount of a functional material having a retrovirus binding domain as well as a target cell binding domain derived from a fibroblast growth factor, a collagen or a polylysine or a functional equivalent thereof on the same molecule;
(b) an artificial substrate for incubating target cells contacted with a retrovirus; and
(c) a target cell growth factor for pre-stimulating the target cells.

For practicing any method of the first and fifth aspects, any culture medium of the second and sixth aspects, any method of the third and seventh aspects and any kit of the fourth and eighth aspects of the present invention, the functional materials immobilized on beads can be suitably used.

The ninth aspect of the present invention relates to a method for improving the gene transfer efficiency into target cells with a retrovirus and characterized in that the target cells are infected with the retrovirus in the presence of an effective amount of a functional material immobilized on beads selected from substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof to permit transduction of the target cells with the retrovirus.

The tenth aspect of the present invention also relates to a method for improving the gene transfer efficiency into target cells with a retrovirus and characterized in that the target cells are infected with the retrovirus in the presence of an effective amount of a functional material selected from substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof without immobilization to permit transduction of the target cells with the retrovirus.

In the above conventional methods as disclosed in WO 95/26200 and Nature Medicine, it is an essential mechanism for improving the gene transfer efficiency with a retrovirus that the retrovirus and the target cells should be co-localized on a functional material having a retrovirus binding domain and a target cell binding domain on the same molecule. In these methods, the co-localization of both retrovirus and target cells on the functional material having both retrovirus binding domain and target cell binding domain on the same molecule firstly becomes possible by immobilizing the functional material having the retrovirus binding domain and the target cell binding domain on the same molecule on a culture substrate.

However, according to the present invention, even when substantially pure fibronectin, a substantially pure fibronectin fragment or a mixture thereof is used, unexpectedly, the gene transfer efficiency into target cells with a retrovirus can be efficiently improved by using the functional material having both retrovirus binding domain and target cell binding domain on the same molecule without immobilization on a culture substrate.

As the target cells to be used in the first, fifth, ninth and tenth aspects of the present invention, there can be used, for example, cells selected from stem cells, hematopoietic cells, non-adherent low density mononuclear cells, adherent cells, bone marrow cells, hematopoietic stem cells, peripheral blood stem cells, umbilical blood cells, fetal hematopoietic stem cells, embryoplastic stem cells, embryonic cells, primordial germ cells, oocyte, oogonia, ova, spermatocyte, sperm, CD 34 + cells, C-kit + cells, multipotential hemopoietic progenitor cells, unipotential hemopoietic progenitor cells, erythrocytic precursor cells, lymphocytic precursor cells, mature blood cells, lymphocytes, B cells, T cells, fibroblast, neuroblast, nerve cells, endothelial cells, angio-endothelial cells, hepatic cells, myoblast, skeletal muscle cells, smooth muscle cells, cancer cells, myeloma cells and leukemia cells.

As the retrovirus to be used in the first, third, fifth, seventh, ninth and tenth aspects of the present invention, a retrovirus containing an exogenous gene can be used and the retrovirus may be, for example, a recombinant retroviral vector. Further, the retrovirus may be, for example, a replication deficient recombinant retroviral vector.

The eleventh aspect of the present invention relates to transduced cells obtained by the first, fifth, ninth or tenth aspect of the present invention.

of target cells in gene transfer to target cells with a retrovirus. The amount can be selected depending upon a particular functional material, a retrovirus and a particular kind of target cells by using the method described herein. The term "the gene transfer efficiency" used herein means the transformation efficiency.

The capability of binding to retroviruses of the functional material, i.e., effectiveness and usefulness of the functional material in the present invention can be ascertained by routine assays as disclosed in Examples hereinafter.

These assays determine the extent to which retrovirus particles are bound to the functional material immobilized to the matrix to be used in the present invention so as to resist washing from the matrix. Briefly, for example, a virus-containing supernatant can be incubated in a well containing the immobilized functional material having a retrovirus binding domain. The well is then thoroughly washed with a physiological saline buffer and thereafter, target cells are incubated in the well to determine the level of infectious activity of the virus remaining in the well. The reduction in infectious activity, or titer, relative to the initial viral supernatant is assessed and compared to that of a similar control (e.g. using a BSA-coated well). A significantly higher titer remaining in the functional material containing well as compared to the control well indicates that the material can be used as the functional material in the present invention.

To facilitate this screening procedure, the viral vector can contain a selectable marker gene.

The functional material having retrovirus binding domain to be used in the present invention can be screened by these assays.

As such a functional material having retrovirus binding domain, there is a functional material which has a retrovirus binding domain derived from Heparin-II binding domain of fironectin, a fibroblast growth factor, a collagen or a polylysine.

The binding of a cell binding domain of the functional material to be used in the present invention to cells, i.e., binding of a material containing a target cell binding ligand to cells can likewise be assayed using conventional procedures. For example, such procedures include those described in Nature 352: 438-441 (1991).

Briefly, the functional material having cell binding domain is immobilized on a culture plate and the cell population to be assayed is overlaid in a medium, followed by incubation for 30 minutes to 2 hours. After this incubation period, cells non-adherent to the functional material are retrieved, counted and assayed for viability. Cells adherent to the functional material are also retrieved using trypsin or cell dissociation buffer (e.g. Gibco), counted and viability tested. In some cases, for example for hematopoietic colony forming cells, the cells are further cultured for an additional 12 to 14 days to ascertain the colony forming characteristics of the cells. The percentage of adherent cells is then calculated and compared to a standard or standard control such as bovine serum albumin (BSA) immobilized on a culture plate. Substantial binding of the target cells to the assayed functional substance provides an indication that the functional material/cell combination is suitable for the present invention and the functional material having cell binding domain can coexist with or be coupled to the functional material having retrovirus binding domain, followed by assessing retrovirus infection of the target cells to construct the functional material to be used in the present invention.

As the functional material having retrovirus binding domain which can be used in the present invention, as described above, there is a functional material which has a retrovirus binding domain derived from Heparin-II binding domain of fironectin, a fibroblast growth factor, a collagen or a polylysine. All substances which have a retrovirus binding domain equivalent to the above and can improve the gene transfer efficiency into target cells with retroviruses by coupling to or co-existing with a ligand having target cell binding domain are included in the functional equivalents to the retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine.

The effective amount of the functional material(s) to be used in the present invention can be determined by using target cells and a retrovirus in the gene transfer method of the present invention in the presence of the selected functional material having retrovirus binding domain coupled to or coexisting with the functional material having target cell binding domain and assessing improvement of the gene transfer efficiency according to the above-described method.

Hereinafter, the present invention will be illustrated in detail.

One aspect of the present invention is a method for improving the gene transfer efficiency into target cells with a retrovirus. This method is characterized by infecting viable target cells with a retrovirus in the presence of a mixture of the functional material having retrovirus binding domain and the functional material having target cell binding domain which is effective for improving the gene transfer efficiency into the target cells with the retrovirus.

This method can be used for obtaining transformant cells transduced with the retrovirus and grafting the cells into an individual organism permits gene transfer into an individual organism.

The functional material having retrovirus binding domain to be used in this method is not specifically limited and examples thereof include Heparin-II binding domain of fibronectin, a fibroblast growth factor, a collagen, a polylysine and the like. Likewise, functional equivalents thereof, for example a functional material having a heparin binding domain can also be used. In addition, a mixture of the functional materials, a polypeptide containing the functional material, a polymer of the functional material, a derivative of the functional material and the like can also be used. These functional materials can be obtained from naturally occurring products, or artificially produced (e.g., produced by genetic engineering techniques or chemical syntheses). Further, they can be produced by combining naturally occurring products with artificial products.

In addition, functional equivalents of the erythropoietin and polypeptides containing erythropoietin or functional equivalents thereof can also be used.

As described in Examples hereinafter, when the functional material having retrovirus binding activity (e.g., H-271 and a fibroblast growth factor) is used in admixed with C-274 which is a polypeptide having a cell binding activity derived from fibronectin or the like, the high gene transfer efficiency can be obtained. NIH/3T3 cells which are used in these experiments express VLA-5 receptor which can bind to C-274 and the interaction of them contribute to improvement of the gene transfer efficiency.

Further, the same phenomenon is also observed, when an erythropoietin derivative is present in gene transfer into TF-1 cells which express erythropoietin receptor (Blood, Vol. 73, pp. 375-380 (1989)). Moreover, this effect is not observed in cells which do not have any erythropoietin receptor.

From these results, it is clear that cell specific increase in the gene transfer efficiency takes place in the presence of the functional material having retrovirus binding domain together with the functional material having cell binding domain.

In this aspect of the present invention, the functional material having retrovirus binding domain is used in the form of a mixture with another functional material having target cell binding domain. Thereby, the gene transfer efficiency into target cells having affinity to the functional materials is remarkably improved. Since the gene transfer efficiency is improved, co-culture with virus producer cells can be avoided and this is one of advantages of the present invention.

Means for selective gene transfer into target cells has high utility and various studies have been done. For example, there is non-viral vector (molecular conjugation vector) wherein a material binding to a receptor present on a cell surface is coupled to a DNA binding material. Examples of gene transfer using such a vector include gene transfer into hepatoma cells with asialoglycoprotein (J. Biol. Chem., Vol. 262, pp. 4429-4432 (1987)), gene transfer into lymphoblasts with transferrin (Proc. Natl. Acad. Sci. USA, Vol. 89, pp. 6099-6103 (1992)), gene transfer into cancer cells with anti EGF receptor antibody (FEBS Letters, Vol. 338, pp. 167-169 (1994)) and the like. These gene transfer methods using non-viral vectors are undesirable from the viewpoint of long term gene expression of transferred genes because the transferred genes are not integrated into chromosomal DNA of cells. Activities have been attempted to use retroviruses which are widely used as vectors capable of insertion of genes into chromosomes to infect specific cells. For examples, gene transfer into hepatocytes by direct chemical modification of retroviruses to couple to lactose (J. Biol. Chem., Vol. 266, pp. 14143-14146 (1991)), gene transfer into erythropoietin receptor-expressing cells by utilizing recombinant viral particles having an envelope protein which is a fused protein with erythropoietin (Science, Vol. 266, pp. 1373-1376 (1994)) and the like have been developed. However, for this purpose, it is necessary to prepare special protein particles according to particular target cells. In addition, chemical modification of viral particles requires complicated procedures and is liable to inactivate viruses. Moreover, regarding a virus envelope modified by gene engineering, the desired product having required functions (binding to target cells and construction of viral particles) is not always obtained.

The above WO 95/26200 suggests that a retroviral vector without any special modification can be transferred into cells in the presence of a fibronectin fragment to which a suitable ligand having cell binding activity is covalently coupled. However, this method uses a functional molecule having both virus binding activity and cell binding activity and therefore an individual special functional material should be prepared according to particular kinds of cells. In addition, it is unknown whether or not the functional material prepared maintains both activities.

The combination of the functional material having retrovirus binding domain and the different functional material having target cell binding domain of the present invention can provide a gene delivery system using retroviruses for a wide variety of cell species. For this purpose, the functional material having retrovirus binding domain does not need to be covalently coupled to the functional material having cell binding domain. Therefore, there is no need to prepare an individual special functional material wherein the functional material having retrovirus binding domain is covalently coupled to the functional material having cell binding domain according to particular kinds of cells and gene transfer into target cells can be conveniently and efficiently carried out.

Examples of gene transfer into target cells using the method of the present invention is gene transfer into cells of the hematopoietic system. It has been known that the above CS-1 cell adhesion region of fibronectin is useful for gene transfer into hematopoietic stem cells. Further, it has also been known that, in addition to the above erythropoietin, various other cell specific cytokines are concerned in differentiation of hematopoietic cells, and gene transfer can be carried out specifically into target cells (cell lines) by utilizing them. For example, when G-CSF is used, megakaryoblasts and granulocytic precursor cells can be used as the target cells of transduction.

When using a substance which specifically or predominantly binds to malignant cells as the functional material having cell binding domain, gene transfer into such target cells can be carried out.

For example, it has been known that receptors named as HER-2 and HER-4 are expressed in certain breast carcinoma cells (Proc. Nat. Acad. Sci. USA, Vol. 92, pp. 9747-9751 (1995)). Accordingly, it is possible to control growth of breast carcinoma cells by combining heregulin which is a ligand for the receptors with the functional material having retrovirus binding domain.

No. 8 and has CS-1 cell adhesion domain derived from fibronectin can be prepared as follows. A DNA fragment is isolated by amplifying by PCR using the above plasmid pCH102 which is prepared from E. coli deposited under Budapest Treaty with NIBH of 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken, Japan, under the accession number of FERM BP-2800 (date of original deposit: May 12, 1989) as a template and the primers CS1-S (the nucleotide sequence is represented by SEQ. ID No. 9 of the Sequence Listing) and M4, and then digesting with the restriction enzymes NheI and SalI.

On the other hand, a DNA fragment is isolated by amplifying by PCR using the plasmid pTF7520ColV, which contains a gene encoding C277-ColV and prepared from above E. coli FERM BP-5277 as a template and the primers CF and CNR, and then digesting with the restriction enzymes AccIII and NheI. The nucleotide sequences of CF and CNR are represented by SEQ. ID Nos. 10 and 12 of the Sequence Listing. The above two DNA fragments are mixed and ligated with an about 4.4 kb DNA fragment obtained by digesting the plasmid pTF7520ColV with the restriction enzymes AccIII and SalI. The resultant plasmid encodes the polypeptide C-ColV-CS1 which has CS-1 cell adhesion domain at the C-terminal of C277-ColV and in which the second glutamic acid from the C-terminal of ColV and the C-terminal threonine are replaced by alanine and serine, respectively. After culture of E. coli transformed with this plasmid, the desired polypeptide can be obtained from the culture. This C-ColV-CS1 is particularly useful in gene transfer into a target cell having CS1 binding property, especially, stem cells.

As the polylysine, as described above, that having a suitable polymerization degree can be selected from commercially available polylysines and used.

The functional materials to be used in the present invention can include derivatives of the above functional materials. Examples thereof include the above C-FGF-CS1 or its functional equivalents and C-ColV-CS1 or its functional equivalents. In addition, polymers obtained by polymerizing plural molecules of the functional materials and modified materials obtained by modifying the functional materials according to known methods (addition of sugar chain, etc.) can also be used in the present invention. These polymers and their functional equivalents can be prepared by genetic engineering techniques using genes encoding the polymers and genes encoding their functional equivalents. In addition, a cysteine-added functional material useful for preparing a polymer of the functional material can be prepared by addition, insertion and substitution of cysteine in the amino acid sequence of the functional material. In addition, a molecule which is a cysteine-added functional material and has a retrovirus binding domain is readily coupled to an another molecule which is a cysteine-added functional material and has a target cell binding domain. Furthermore, a material coupled to other functional material can be prepared by utilizing the reactivity of the cysteine residue of the cysteine-added functional material.

In another preferred aspect of the present invention, gene transfer is carried out by using a polymer of the retrovirus binding domain of fibronectin which increases the gene transfer efficiency into target cells with retroviruses.

The functional material is a polypeptide having plural Heparin-II binding domains of human fibronectin in one molecule as described in the above WO 95/26200 or derivatives of the polypeptide. In so far as the same activity as that of the functional material is maintained, functional equivalents a part of whose amino acid sequences are different from that of the naturally occurring products can be included.

Examples of the polymer of the functional material include those obtained by enzymologically or chemically polymerizing the above polypeptide derived from fibronectin or by gene engineering techniques. An example of a polypeptide which has two Heparin-II binding domains derived from fibronectin in a molecule include a polypeptide having an amino acid sequence represented by SEQ. ID No. 13 of the Sequence Listing (hereinafter referred to as H2-547). H2-547 can be obtained according to the method described herein by using E. coli which has been deposited under Budapest Treaty with NIBH of 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken, Japan, under the accession number of FERM BP-5656 (date of original deposit: September 6, 1996). A polypeptide having an amino acid sequence represented by SEQ. ID No. 14 of the Sequence Listing is a polypeptide derivative containing a cell adhesion polypeptide of fibronectin coupled at the N-terminal of H2-547 (hereinafter referred to as CH 2-826). This polypeptide can be obtained according to the method disclosed herein. Further, a polypeptide having an amino acid sequence represented by SEQ. ID No. 30 of the Sequence Listing is a polypeptide derivative containing CS-1 cell adhesion region of fibronectin coupled at the C-terminal of H2-547 (hereinafter referred to as H2S-573). The polypeptide can be obtained according to the method described herein by using E. coli which has been deposited under Budapest Treaty with NIBH of 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken, Japan, under the accession number of FERM BP-5655 (date of original deposit: September 6, 1996). H2S-573 having CS-1 cell adhesion region is useful for gene transfer into hematopoietic stem cells.

In yet another preferred aspect of the present invention, viable target cells are infected with a replication deficient retroviral vector in the presence of the functional material immobilized on beads which is effective to increase the gene transfer efficiency into cells with a retroviral vector.

Conventional methods for improving the gene transfer efficiency into target cells with a retroviral vector by using the functional materials described in the above WO 95/26200 and Nature Medicine are carried out by immobilizing the functional materials on a vessel to be used for infection of cells with viruses (a plate for cell culture). These methods require complicated procedures such as washing of excess functional material after treatment of the plate with a solution containing the functional material.

Moreover, the method of the present invention is suitable for protocols of clinical gene therapy because co-cultivation of target cells in the presence of retrovirus producer cells is not required and the method of the present invention can be carried out in the absence of hexadimethrine bromide whose use is clinically disadvantageous in human being.

Further, as application of the present invention to art fields other than gene therapy, for example, transgenic vertebrate animals can be simply produced by using, as a target cells, embryoplastic stem cells, primordial germ cell, oocyte, oogonia, ova, spermatocyte, sperm and the like.

That is, as one aspect, the present invention provides a method for cellular grafting comprising grafting the transformant cells obtained by the method of the present invention into a vertebrate animal. Examples of vertebrate animals to be grafted with transformant cells include mammals (e.g., mouse, rat, rabbit, goat, pig, horse, dog, monkey, chimpanzee, human being, etc.), birds (e.g., chicken, turkey, quail, duck, wild duck, etc.), reptiles (e.g, snake, alligator, tortoise, etc.), amphibian (e.g., frog, salamander, newt, etc.), fishes (e.g., dog mackerel, mackerel, bass, snapper, grouper, yellowtail, tuna, salmon, trout, carp, sweetfish, eel, flounder, shark, ray, sturgeon, etc.).

Thus, according to this aspect of the present invention, like substantially pure fibronectin, substantially pure fibronectin fragments or a mixture thereof, gene transfer with retroviruses can be carried out efficiently by the retrovirus binding domain and the target cell binding domain of the functional material to be used in the present invention. Then, the present invention can provide a technique for transferring genetic materials into vertebrate cells without any limitation of conventional techniques.

In a further aspect of the present invention, an effective amount of a material which has both retrovirus binding domain and target cell binding domain on the same molecule and has functions equivalent to those of substantially pure fibronectin, substantially pure fibronectin fragments or a mixture thereof is used as the functional material.

Such a functional material is a material which can perform gene transfer with the same efficiency as that of fibronectin, a fibronectin fragment or a mixture thereof. Typically, it is the functional material having the above novel retrovirus binding domain and target cell binding domain of the present invention on the same molecule. In case of using these materials, it is considered that retroviruses as well as target cells bind to at least one functional material.

Examples of the functional material having a retrovirus binding domain and a target binding domain on the same molecule include polypeptides represented by SEQ. ID Nos. 21 and 22 of the Sequence Listing (hereinafter referred to as CHV-181 and CHV-179, respectively).

These peptides include type III similar sequences (III-12, III-13 and III-14) contained in H-271. In CHV-181, III-12 and III-13 sequences, and in CHV-179, III-13 and III-14 sequences are added to the C-terminal of the cell adhesion polypeptide (Pro^{1239}-Ser^{1515}) of fibronectin via methionine. A plasmid for expressing the polypeptide CHV-181 can be constructed, for example, by the following procedures.

First, the plasmid pHD101 containing a DNA fragment encoding the heparin binding polypeptide (H-271) of fibronectin is prepared in Escherichia coli HB101/pHD101 (FERM BP-2264). A HindIII site is introduced in a region encoding the C-terminal of the III-13 sequence on this plasmid by site-directed mutagenesis, followed by digestion with NcoI and HindIII to obtain a DNA fragment encoding III-12 and III-13 sequence. On the other hand, the plasmid vector pINIII-$ompA_1$ is digested with HindIII and SalI to obtain a DNA fragment encoding a lipoprotein terminator region.

Next, the plasmid pTF7021 containing a DNA fragment encoding the cell adhesion polypeptide (C-279) of fibronectin is prepared from Escherichia coli JM109/pTF7021 (FERM BP-1941), and a NcoI site is introduced immediately before termination codon of C-279 on the plasmid by site-directed mutagenesis to obtain the plasmid pTF7520. This plasmid is digested with NcoI and SalI, followed by mixing with the DNA fragment encoding the III-12 and III-13 sequence and the DNA fragment encoding a lipoprotein terminator region to ligate them to obtain the plasmid pCHV181 for expressing the polypeptide CHV-181. The nucleotide sequence of a region encoding the polypeptide CHV-181 on the plasmid pCHV181 is shown in SEQ. ID No. 27 of the Sequence Listing.

A plasmid for expressing the polypeptide CHV-179 can be constructed, for example, by the following procedures.

First, a NcoI site is introduced in a region encoding the N-terminal of the III-13 sequence on the plasmid pHD101 by site-directed mutagenesis, followed by digestion with NcoI and HindIII to obtain a DNA fragment encoding the III-13 and III-14 sequence. This is mixed with a DNA fragment encoding the above lipoprotein terminator region and the NcoI and SalI-digested plasmid pTF7520 to ligate them to obtain the plasmid pCHV179 for expressing the polypeptide CHV-179.

CHV-181 and CHV-179 can be obtained by culturing E. coli transformed with the above plasmids, respectively, then purifying from the resulting culture.

These functional materials can be used by immobilized on, for example, beads as described above or without immobilization.

In another aspect, the present invention provides a culture medium of target cells to be used for gene transfer into the target cells with retroviruses which comprises (1) the above-described mixture of an effective amount of the functional material having retrovirus binding domain and an effective amount of another functional material having the target cell binding domain or (2) an effective amount of the functional material having the above described novel retrovirus binding domain and target cell binding domain on the same molecule. The functional material may be immobilized or

The fragments of fibronectin described herein may be of natural or synthetic origin and can be prepared in substantial purity from naturally occurring materials, for example as previously described by Ruoslahti et al. (1981) J. Biol. Chem. 256:7277; Patel and Lodish (1986) J. Cell. Biol. 102:449; and Bernardi et al. (1987) J. Cell. Biol. 105:489. In this regard, reference herein to substantially pure fibronectin or a fibronectin fragment is intended to mean that they are essentially free from other proteins with which fibronectin naturally occurs.

The substantially pure fibronectin or fibronectin fragment described herein can also be produced by genetic engineering techniques, for example, as generally described in U.S. Patent No. 5,198,423. In particular, the recombinant fragments identified in the Examples below as H-271, H-296, CH-271 (SEQ ID NO 23) and CH-296 (SEQ ID NO 24), and methods for obtaining them, are described in detail in this patent. The C-274 fragment used in the Examples below was obtained as described in U.S. Patent No. 5,102,988. These fragments or fragments from which they can be routinely derived are available by culturing E. coli deposited with NIBH of 1-1-3, Higashi, Tsukuba-sh, Ibaraki-ken, Japan under Budapest Treat with the accession numbers of FERM P-10721 (H-296) (the date of original deposit: May 12, 1989), FERM BP-2799 (C-277 bound to H-271 via methionine) (the date of original deposit: May 12, 1989), FERM BP-2800 (C-277 bound to H-296 via methionine) (the date of original deposit: May 12, 1989) and FERM BP-2264 (H-271) (the date of original deposit: January 30, 1989), as also described in U.S. Patent No. 5,198,423.

In addition, useful information as to fibronectin fragments utilizable herein or as to starting materials for such fragments may be found in Kimizuka et al., J. Biochem. 110, 284-291 (1991), which reports further as to the above-described recombinant fragments; in EMBO J., 4, 1755-1759 (1985), which reports the structure of the human fibronectin gene; and in Biochemistry, 25, 4936-4941 (1986), which reports on the Heparin-II binding domain of human fibronectin. Fibronectin fragments which contain both the CS-I cell adhesion domain and the Heparin-II binding domain have been found to significantly enhance the efficiency of gene transfer into hematopoietic cells in work thus far.

It will thus be understood that the fibronectin-related polypeptides described herein will provide an amino acid sequence having the cell-binding activity of the CS-I cell adhesion domain of fibronectin as well as an amino acid sequence of the Heparin-II binding domain of fibronectin which binds the virus.

The viral-binding polypeptide utilized to enhance transduction by retroviral vectors as disclosed in WO 95/26200 will comprise (i) a first amino acid sequence which corresponds to the Ala^{1690} - Thr^{1960} of the Heparin-II binding domain of human fibronectin, which is represented by the formula (SEQ ID NO 1):

Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Sys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Sys Pro Gly Sev Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys Thr;

or a sufficiently similar amino acid sequence thereto to exhibit the ability to bind the retrovirus;

and (ii) a second amino acid sequence (CS-1) which corresponds to one portion of the IIICS binding domain of human fibronectin; which is represented by the formula (SEQ. ID No. 2):

Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr;

or a sufficiently similar amino acid sequence thereto to exhibit the ability to bind hematopoietic cells such as primitive progenitor and/or long term repopulating (stem) cells.

The retrovirus binding activity of a polypeptide represented by the above SEQ. ID No. 1 (H-271) shows a concentration dependence and, as indicated in Example 8 below, it shows substantially the same activity as that of CH-271 at high concentrations. That is, a retrovirus and target cells bind to at least one molecule of H-271 for the first time in the presence of a high concentration of H-271.

The strong virus binding to the virus binding domain of the functional material of the present invention can be used for constructing delivery systems for virus-mediated therapy across a broad range of cell types. For this purpose, a polypeptide containing the retrovirus binding domain of the functional material of the present invention can be coupled to any material containing a cell binding domain which gives this construct specificity for the target cells, or can be co-localized with a material containing its cell binding domain. That is, the virus binding polypeptide may be covalently coupled to the cell binding material or they may be different molecules.

This approach will circumvent the prior necessity of constructing specific retrovirus cell lines for each target cell and facilitate selection of the functional material having the most suitable target cell binding domain according to a particular kind of target cells. Therefore, by using the functional material of the present invention, transduction specific for target cells to be used can be readily carried out and, in particular, the method of the present invention wherein a mixture of the functional material having retrovirus binding domain and the functional material having target cell binding domain is

(2) Preparation of C-FGF • A

The polypeptide, C-FGF • A (amino acid sequence is shown in SEQ. ID No. 4 of the Sequence Listing) was prepared as follows. Namely, E. coli containing the recombinant plasmid containing DNA encoding the above polypeptide, pYMH-CF • A, i.e., Escherichia coli JM109/pYMH-CF • A (FERM BP-5278) was cultured in 5 ml of LB broth containing 100 μg/ml of ampicillin at 37°C for 8 hours. This pre-culture broth was inoculated into 500 ml of LB broth containing 100 μg/ml of ampicillin and 1 mM of IPTG (isopropyl-β-D-thiogalactopyranoside) and cultivated at 37°C overnight. The microbial cells were harvested, suspended in 10 ml of PBS (phosphate buffered saline) containing 1 mM PMSF (phenylmethanesulfonium fluoride) and 0.05% of Nonidet P-40 and sonicated to disrupt the cells. The mixture was centrifuged to obtain a supernatant. To absorbance 4,000 at 260 nm of this supernatant was added 1 ml of 5% polyethylene imine and the mixture was centrifuged to obtain a supernatant. The supernatant was applied to a HiTrap-Heparin column (Pharmacia) equilibrated with PBS. After washing the non-absorbed fraction with PBS, the absorbed fraction was eluted with PBS containing NaCl gradient of from 0.5 M to 2 M. The eluate was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) which showed the presence of two fractions containing 47 kd polypeptide. One fraction of them which was eluted at the higher NaCl concentration was collected and applied to a Superose 6 column (Pharmacia) equilibrated with PBS containing 1.5 M NaCl. The eluate was analyzed by SDS-PAGE and a fraction containing about 47 kd polypeptide was collected to obtain the purified C-FGF • A which was used in the subsequent steps.

(3) Preparation of C-FGF-CS1

First, a plasmid was constructed for expressing the polypeptide, C-FCF-CS1 (amino acid sequence is shown in SEQ. ID No. 5 of the Sequence Listing) in Escherichia coli as a host.

Escherichia coli HB101/pCH102 (FERM BP-2800) was cultured and the plasmid pCH102 was prepared by alkali-SDS method from the resulting microbial cells. PCR was carried out using this plasmid as a template as well as primer M4 (Takara Shuzo Co., Ltd.) and primer CS1-S, nucleotide sequence of which is shown in SEQ. ID No. 9 in the Sequence Listing and an amplified DNA fragment in the reaction solution was recovered with ethanol precipitation. The resultant DNA fragment was digested with NheI and SalI (both Takara Shuzo Co., Ltd.), followed by agarose gel electrophoresis to recover about 970 bp DNA fragment from the gel.

Escherichia coli JM109/pYMH-CF • A (FERM BP-5278) was then cultured and the plasmid pYMH-CF • A was prepared by an alkali-SDS method from the resulting microbial cells. PCR reaction was carried out using this plasmid as a template as well as primer CF, nucleotide sequence of which is shown in SEQ. ID No. 10, and primer FNR, nucleotide sequence of which is shown in SEQ. ID No. 11 of the Sequence Listing, and an amplified DNA fragment in the reaction solution was recovered with ethanol precipitation. The resultant DNA fragment was digested with Eco52I (Takara Shuzo Co., Ltd.) and NheI, followed by agarose gel electrophoresis to recover about 320 bp DNA fragment from the gel.

About 4.1 kb DNA fragment isolated by digesting the plasmid pYMH-CF • A with Eco52I and SalI and subjecting to agarose gel electrophoresis was mixed with the above 970 bp DNA fragment and about 320 bp DNA fragment to ligate them to obtain a recombinant plasmid which was inserted into E. coli JM109. A plasmid was prepared from the resulting transformant and that containing each one molecule of the above three DNA fragments was selected and named plasmid pCFS100. E. coli JM109 transformed with the plasmid pCFS100 was named Escherichia coli JM109/pCRS100. The plasmid pCFS100 has a CS-1 cell adhesion region derived from fibronectin at the C-terminal of C-FGF • A and encodes the polypeptide, C-FGF-CS1, wherein second lysine from the C-terminal of FGF was substituted with alanine.

The polypeptide, C-FGF-CS1 was prepared as follows. Namely, the above E. coli JM109/pCFS100 was cultured in 5 ml of LB broth containing 100 μg/ml of ampicillin at 37°C for 8 hours. This pre-cultured broth was inoculated into 500 ml of LB broth containing 100 μg/ml of ampicillin and 1 mM IPTG and cultured overnight at 37°C to collect the microbial cells. The resulting microbial cells were suspended in 10 ml of PBS (phosphate buffered saline) containing 0.5M NaCl, 1mM PMSF and 0.05% Nonidet P-40, and the microbial cells were sonicated to disrupt and centrifuged to obtain a supernatant. This supernatant was subjected to HiTrap-Heparin column pre-equilibrated with PBS containing 0.5 M NaCl, the non-adsorbed fractions were washed with PBS containing 0.5 mM NaCl and the adsorbed fraction was eluted with PBS having a concentration gradient of 0.5 M to 2 M NaCl. The eluate was analyzed by SDS-polyacrylamide gel electrophoresis and fractions containing about 50 kd polypeptide were collected to obtain purified C-FGF-CS1 which was used in the subsequent steps.

Amino acid sequence of from N-terminal to the fifth amino acid of purified C-FGF-CS1 thus obtained was investigated and found to be consistent with that shown in SEQ. ID No. 5 of the Sequence Listing. In addition, molecular weight of purified C-FGF-CS1 measured by masspectroscopy was consistent with that expected from the above amino acid sequence.

nected in tandem, and lipoprotein terminator in this order. A nucleotide sequence of the above open reading frame is shown SEQ. ID No. 17 of the Sequence Listing.

The polypeptide, H2-547 was prepared as follows. Four 500 ml Erlenmeyer flasks, equipped with a baffle, containing 120 ml of LB broth containing 100 μg/ml of ampicillin were prepared, these were inoculated with E. coli HB101 transformed with the above plasmid pRH2-T, that is, Escherichia coli HB101/pRH2-T to culture overnight at 37°C. The microbial cells were collected from the culture by centrifugation, suspended in a 40 ml disruption buffer (50 mM tris-HCl, 1 mM EDTA, 150 mM NaCl, 1 mM DTT, 1 mM PMSF, pH 7.5) and the microbial cells were sonicated to disrupt. The supernatant obtained by centrifugation was subjected to High trap Heparin column (Pharmacia) equilibrated with a purification buffer (50 mM tris-HCl, pH 7.5). The non-adsorbed fractions in the column were washed with the same buffer, followed by elution with a purification buffer having the concentration gradient of 0 to 1 M NaCl. The eluate was analyzed with SDS-polyacrylamide gel electrophoresis and the fractions containing a polypeptide having the molecular weight of about 60,000 were collected to obtain purified H2-547 preparation. The protein amount contained in the resulting preparation was analyzed with BCA PROTEIN ASSAY REAGENT (Pierce) using bovine serum albumin as a standard, indicating that about 10 mg of H2-547 was obtained.

Amino acid sequence of from the N-terminal to the fifth residue of purified H2-547 thus obtained was investigated and found to be consistent with amino acid sequence of H2-547 expected from nucleotide sequence shown in SEQ ID NO 17 of the Sequence Listing minus methionine at the N-terminal (sequence thereof is shown in SEQ. ID No. 13 of the Sequence Listing). The molecular weight of purified H2-547 measured by masspectroscopy was consistent with that expected from amino acid sequence shown in SEQ. ID No. 13 of the Sequence Listing.

(7) Preparation of CH2-826

A plasmid for expressing the polypeptide, CH2-826 (amino acid sequence is shown in SEQ. ID No. 14 of the Sequence Listing) was constructed as follows. PCR was carried out using the above plasmid pCH102 as a template as well as primer CLS, the nucleotide sequence of which is shown in SEQ. ID No. 18 of the Sequence Listing, and primer CLA, the nucleotide sequence of which is shown in SEQ. ID No. 19 of the Sequence Listing, followed by agarose gel electrophoresis to recover an about 0.8 kb DNA fragment encoding the cell adhesion polypeptide of fibronectin. The resulting DNA fragment was digested with NcoI and BglII (both Takara Shuzo Co., Ltd.) and mixed with NcoI-BamHI digested pTV118N to ligate them, which was inserted into E. coli JM109. Plasmids were prepared from the resulting transformant and a plasmid containing the above DNA fragment was selected and named plasmid pRC1. An about 2.5 kb DNA fragment obtained by digesting this plasmid pRC1 with SpeI and ScaI and an about 3.9 kb DNA fragment obtained by digesting the above plasmid pRH2-T with NheI and ScaI were mixed to ligate them to obtain the plasmid pRCH2-T encoding a polypeptide wherein two heparin binding polypeptides are tandemly connected to the C-terminal of the cell adhesion polypeptide. A nucleotide sequence of open reading frame on the plasmid pRCH2-T encoding this polypeptide is shown in SEQ. ID No. 20 of the Sequence Listing.

The polypeptide, CH2-826 was prepared according to the same method as that used for the polypeptide H2-547 described in Example 2 (6). The fractions containing a polypeptide having the molecular weight of about 90,000 were collected from the eluate of High trap Heparin column to obtain purified CH2-826.

(8) Preparation of H2S-537

A plasmid for expressing the polypeptide, H2S-537 (amino acid sequence is shown in SEQ. ID No. 30 of the Sequence Listing) was constructed as follows. PCR was carried out using the above plasmid pCH102 as a template as well as primer CS1S, the nucleotide sequence of which is shown in SEQ. ID No. 31 of the Sequence Listing, and primer CS1A, the nucleotide sequence of which is shown in SEQ. ID No. 32 of the Sequence Listing, followed by agarose gel electrophoresis to recover an about 0.1 kb DNA fragment encoding the cell adhesion polypeptide of fibronectin. The resulting DNA fragment was digested with NcoI and BamHI (both Takara Shuzo Co., Ltd.) and mixed with NcoI-BamHI digested pTV118N to ligate them, which was inserted into E. coli JM109. Plasmids were prepared from the resulting transformant and a plasmid containing the above DNA fragment was selected and named plasmid pRS1.

The plasmid vector, pINIII-ompA$_1$ was digested with BamHI and HincII to recover an about 0.9 kb DNA fragment containing a lipoprotein terminator region. This was mixed with BamHI-HincII digested plasmid pRS1 to ligate them to obtain the plasmid pRS1-T containing lac promoter, DNA fragment encoding CS-1 region polypeptide and lipoprotein terminator in this order.

An about 2.4 kb DNA fragment obtained by digesting this plasmid pRS1-T with NheI and ScaI and an about 3.3 kb DNA fragment obtained by digesting the above plasmid pRH2-T with SpeI, ScaI and PstI (Takara Shuzo Co., Ltd.) were prepared. They were ligated to obtain the plasmid pRH2S-T containing lac promoter, open reading frame encoding a polypeptide having such structure in which two heparin binding polypeptides are tandemly connected and CS-1 region is further coupled to the C-terminal thereof, and lipoportein terminator in this order. A nucleotide sequence of the above

has the cell binding domain, the higher gene transfer efficiency can be obtained in comparison with that obtained by using FGF alone and that covalent coupling of the polypeptides is not necessary required for elaborating such effect of the combination of the polypeptides.

(2) Gene transfer using mixture of functional materials

According to the same manner as described in Example 3 (1), assessment was carried out except that the polypeptide having retrovirus binding domain was replaced with ColV. In this experiment, the effect was investigated by mixing C-274 and ColV in various molar ratios. Namely, according to the same manner as described in Example 2 (9), immobilization on plates was carried out by using 330 pmol/cm^2 (6 μg/cm^2) of ColV, a mixture of 330 pmol/cm^2 (10 μg/cm^2) of C-274 and 330 pmol/cm^2 of ColV (molar ratio of C-274 : ColV = 10 : 10), a mixture of 100 pmol/cm^2 (3 μg/cm^2) of C-274 and 330 pmol/cm^2 of ColV (3 : 10), a mixture of 33 pmol/cm^2 (1 μg/cm^2) of C-274 and 330 pmol/cm^2 of ColV (1 : 10), 330 pmol/cm^2 (16 μg/cm^2) of C277-ColV and 330 pmol/cm^2 (10 μg/cm^2) of C-274, respectively. By using the plates thus prepared, the effect of retrovirus infection was investigated according to the same manner as described above. The results are shown in Fig. 3. In Fig. 3, the abscissa indicates the functional materials used and the ordinate indicates the gene transfer efficiency.

As shown in Fig. 3, in case of 2 hour infection, the infection efficiency of ColV immobilized plate was less than 1/2 of that of C277-ColV immobilized plate, while the infection efficiency of the plate on which immobilization was carried out with the mixture of ColV and its 1/10 amount (as the molecular number) of C274 was the same as that of C277-ColV immobilized plate. Then, the retrovirus infection enhancing activity of C-274 was ascertained as observed in the case of FGF. This effect was rather decreased in case that the amount of C-274 molecules relative to ColV molecules was increased. When a mixture containing the same amounts of ColV and C-274 was coated, there was no substantial difference between the mixture and ColV alone.

(3) Gene transfer using mixture of functional materials

In order to investigate the effect on the gene transfer efficiency by immobilization of a mixture of a material having cell binding domain and a material having retrovirus binding domain, the following experiment was carried out. First, according to the same manner as described in Example 2 (9), immobilization of plates was carried out with 32 pmol/cm^2 (1 μg/cm^2) of C-274, 333 pmol/cm^2 (10 μg/cm^2) of H-271 and a mixture of 32 pmol/cm^2 (1 μg/cm^2) of C-274 and 333 pmol/cm^2 (10 μg/cm^2) of H-271, respectively. After pre-incubating 2 ml of a virus supernatant containing 1,000 cfu of PM5neo virus in respective plates at 37°C for 30 minutes, the plates were thoroughly washed with PBS. To each of these plates was added 2 ml of DMEM medium containing 2,000 NIH/3T3 cells and incubated at 37°C for 2 hours. Non adhered cells were collected by decantation and cells adhered to the plate were collected by trypsin treatment to detach them from the plate. The cells were combined. The resultant cell suspension was divided into two halves. One half portion was cultured in DMEM and the other portion was cultured in DMEM containing G418 at a final concentration of 0.75 mg/ml. Both portions were incubated at 37°C for 10 days and the colonies appeared were counted. By taking the ratio of the number of G418^r colonies relative to that obtained in the medium without G418 as the gene transfer efficiency, the results are shown in Fig. 4. In Fig. 4, the abscissa indicates the functional materials used and the ordinate indicates the gene transfer efficiency.

As shown in Fig. 4, when using the plate on which the mixture of C-274 and H-271 (molar ratio = 1 : 10) was immobilized, the infection efficiency was significantly increased. No gene transfer was observed in C-274 immobilized plate.

(4) Gene transfer using C277-CS1

In order to investigate the effect on the infection efficiency by using C277-CS1 as a material having cell binding domain and immobilization of a mixture thereof and a material having retrovirus binding domain, the following experiment was carried out. As the material binding to a retrovirus, a polylysine [$(Lys)_n$, poly-L-lysyine hydrobromide, molecular weight: 50,000-100,000, Wako Pure Chemical Co., Ltd.] and H-271 were used. As the cells, non-adherent cells, TF-1 cells (ATCC CRL-2003), were used. First, according to the same manner as described in Example 2 (9), immobilization on plates was carried out by using the following solutions: C-277-CS1 (33 pmol/cm^2, 1.1 μg/cm^2), polylysine (133 pmol/cm^2, 10 μg/cm^2), a mixture of C-277-CS1 (33 pmol/cm^2) and polylysine (133 pmol/cm^2), H-271, (333 pmol/cm^2, 10 μg/cm^2) and a mixture of C-277-CS1 (33 pmol/cm^2) and H-271 (333 pmol/cm^2) and CH-296 (33 pmol/cm^2, 2.1 μg/cm^2), respectively. To each plate was added RPMI 1640 medium [containing 5 ng/ml of GM-CFS (Petro Tech), 50 units/ml of penicillin and 50 μg/ml of streptomycin] containing 1 x 10^4 cfu of TKNEO virus, 1 x 10^4 of TF-1 cells and the plate was incubated at 37°C for 24 hours. After incubation, non adhered cells were collected by decantation and cells adhered to the plate were collected by trypsin treatment to remove them from the plate. The cells were combined. Respective one fifth portions of the resultant cell suspension were transferred to two plates coated with

(6) Gene transfer into erythropoietin receptor expressing cells

The effect of gene transfer using erythropoietin as a material having cell binding activity was investigated by using two kinds of cells, TF-1 which expresses an erythropoietin receptor and HL-60 (ATCC CCL-240) which does not express the erythropoietin receptor. In this investigation, the above polypeptide derivative of erythropoietin (GST-Epo) was used as erythropoietin and a polylysine was used as the retrovirus biding material. First, according to the same manner as described in Example 2 (9), immobilization on plates was carried out by using GST-Epo corresponding to 34 pmol/cm^2 (1.5 μg/cm^2), polylysine (133 pmol/cm^2, 10 μg/cm^2), a mixture of GST-Epo (34 pmol/cm^2) and polylysine (133 pmol/cm^2), respectively. To each plate was added a medium containing 1 x 10^4 cfu of TKNEO virus and 1 x 10^4 of cells and the plate was incubated at 37°C for 24 hours. As the medium, RPMI1640 medium (containing 5 ng/ml of GM-CFS, 50 units/ml of penicillin and 50 μg/ml of streptomycin) was used for TF-1 and RPMI medium (Nissui, containing 10% FCS, 50 units/ml of penicillin, 50 μg/ml of streptomycin) was used for HL-60. After incubation, non adhered cells were collected by decantation and cells adhered to the plate were collected by trypsin treatment to remove them from the plate. The cells were combined. Respective one fifth portions of the resultant cell suspension were transferred to two CH-296 immobilized plates and incubated for 24 hours. Then, the medium of one portion was exchanged to the above medium and that of the other portion was exchanged to the above medium containing G418 at a final concentration of 0.75 mg/ml. Both portions were incubated at 37°C for 8 days and the colonies appeared were counted. The incidence of G418^r colonies (the gene transfer efficiency) was calculated based on the numbers of colonies appeared in the presence and absence of G418.

The results are shown in Fig. 6. In Fig. 6, the abscissa indicates the functional materials used and the ordinate indicates the gene transfer efficiency, respectively. In case of using TF-1 cells as shown in Fig. 6 (a), although gene transfer was taken place to some extent in the plate on which only the polylysine was immobilized, the higher gene transfer efficiency was obtained in the presence of GST-Epo. On the other hand, in case of using HL-60 as shown in Fig. 6 (b), no increase in the gene transfer efficiency was observed in the presence of GST-Epo. These results showed that target cell specific gene transfer was possible by using erythropoietin.

In addition, an experiment of gene transfer into TF-1 cells was carried out by replacing the retrovirus binding material with H2-547. According to the same manner as described in Example 2 (9), immobilization on plates was carried out by using H2-547 (333 pmol/cm^2, 20 μg/cm^2), GST-Epo corresponding to 34 pmol/cm^2, 1.5 μg/cm^2) and a mixture of GST-Epo (34 pmol/cm^2) and H2-547 (333 pmol/cm^2, 20 μg/cm^2), respectively. At the same time, a control experiment was carried out by using BSA immobilized plate.

The results are shown in Fig. 7. In Fig. 7, the abscissa indicates the functional materials used and the ordinate indicates the gene transfer efficiency, respectively. As shown in Fig. 7, in case of using H2-547, the gene transfer efficiency into TF-1 cells was increased in the presence of GST-Epo.

(7) Gene transfer using beads on which mixture of functional materials was immobilized

Whether the retrovirus infection efficiency can be increased by using beads on which both material having cell binding domain and material having retrovirus binding domain were immobilized or not was investigated.

Beads on which polypeptides were immobilized were prepared according to the following procedures. As beads, polystyrene beads having the diameter of 1.14 μm (Polybeads Polystyrene Microsphere, manufactured by PolyScience) were used. To 20 μl of a 2.5% suspension of the above beads were added 80 μl of ethanol and 2 ml of various polypeptide solutions in PBS, followed by allowing to stand overnight at 4°C. To this were added BSA and PBS to prepared 4 ml of 1% BSA/PBS suspension. Beads were recovered from the suspension by centrifugation and suspended in 5 ml of 1% BSA/PBS again. Then, the suspension was allowed to stand at room temperature for 1 hour to obtain a suspension of polypeptide immobilized beads. As the polypeptide solutions, 100 μg/ml of C-274, 100 μg/ml of H-271, 100 μg/ml of CH-271, 100 μg/ml of CH-296 and a mixture of 100 μg/ml of H-271 and 10 μg/ml of C-274. As a control, beads coated with 2% BSA solution was prepared according to the same manner.

One tenth portion of the polypeptide immobilized beads thus prepared was recovered from the above suspension and incubated at 37°C overnight together with 2,000 of TF-1 cells and 1,000 cfu of TKNEO virus supernatant, respectively. The cells were recovered and suspended in RPMI medium [containing 10% of FCS, 5 ng/ml of GM-CFS (Petrotech), 50 units/ml of penicillin and 50 μg/ml of streptomycin] containing 0.3% of Bacto agar (Difco) and seeded on a 35 mm plate made of the above medium containing 0.5% of Bacto agar. Two mediums containing 0.75 mg/ml of G418 and without G418 were used. The plate was incubated in 5% CO_2 at 37°C for 14 days. Colonies which appeared in the presence of G418 and in the absence of G418 were counted and the appearance ration of G418^r colonies (gene transfer efficiency) was calculated.

The results are shown in Fig. 8. In Fig. 8, the abscissa indicates the functional material used and BSA and the ordinate indicates the gene transfer efficiency. When using the beads on which the mixture of H-271 and C-274 was immobilized, the higher gene transfer efficiency was obtained in comparison with using beads on which only H-271 alone was

following experiment was carried out.

150 mg/kg 5-fluorouracil (5-FU, Amlesco) was administered intraperitoneally to mouse (C3H/HeJ), 6 to 8 weeks age, femur and tibia were isolated 2 days after administration to collect bone marrow. The resulting bone marrow was subjected to density gradient centrifugation using Ficoll-Hypaque (density 1.0875 g/ml, Pharmacia) to obtain a low density mononuclear cell fraction which was used as mouse bone marrow cells.

The mouse bone marrow cells were pre-stimulated prior to infection with retrovirus according to a method by Luskey et al. (Blood, 80, 396 (1992)). Namely, the mouse bone marrow cells were added to α-MEM (Gibco) containing 20% of FCS, 100 units/ml of recombinant human interleukin-6 (rhIL-6, Amgen), 100 ng/ml of recombinant mouse stem cell factor (rmSCF, Amgen), 50 units/ml of penicillin and 50 μg/ml of streptomycin at cell density of 1 X 10^6 cells/ml, followed by incubation at 37 °C for 48 hours in 5% CO_2. The pre-stimulated cells including those adhered to the container were collected by aspiration with a pipette.

Each 2 ml of the medium, used for the above pre-stimulation, containing 1 X 10^6 pre-stimulated cells and 1 X 10^4 cfu of PM5neo virus was added to the plate prepared with 236 pmol/cm^2 (4 μg/cm^2) of FGF, 169 pmol/cm^2 (8 μg/cm^2) of C-FGF • A or 159 pmol/cm^2 (8 μg/cm^2) of C-FGF-CS1 according to the method described in Example 2 (9), and a BSA immobilized plate (control plate), followed by incubation at 37°C. After 2 hours, a medium (2ml) containing the same amount of virus was freshly added to each plate, followed by continuing incubation for 22 hours. After completion of incubation, the non-adhered cells were collected by decantation and the cells adhered to the plate were collected using a cell dissociation buffer (CDB, containing no enzymes, Gibco) and these cells were combined and washed twice with the same buffer. The number of the cells was counted. The collected cells were subjected to HPP-CFC (High Proliferative Potential-Colony Forming Cells) assay.

HPP-CFC assay was carried out according to a method by Bradley et al. (Aust. J. Exp. Biol. Med. Sci., 44, 287-293 (1966)). As a medium, 1%/0.66% layered soft agar medium with or without G418 at the final concentration of 1.5 mg/ml was used. Infected cells was added thereto at 1 X 10^4 cells/well, followed by incubation at 37 °C for 13 days in 10% CO_2. After completion of incubation, the colonies which appeared were observed with an inverted microscope and the number of high density colonies (having the diameter of not less than 0.5 mm) derived from HPP-CFC was counted to calculate the incidence (gene transfer efficiency) of $G418^r$ colonies. The results are shown in Fig. 12. In Fig. 12, the abscissa indicates the functional material used and BSA and the ordinate indicates the gene transfer efficiency.

As shown in Fig. 12, no $G418^r$ colonies appeared in the plate coated with BSA as a control, while the $G418^r$ colonies were obtained when the plates on which the above respective polypeptides were immobilized were used. The gene transfer efficiencies were increased in an order of in FGF, C-FGF • A and C-FGF-CS1, suggesting that the presence of the cell adhesion domain derived from fibronectin and CS-1 polypeptide which has the binding activity to cells domain increase the infection of bone marrow cells with retrovirus.

(5) Relation between concentration of C277-ColV used for immobilization on plate and gene transfer efficiency

The gene transfer efficiencies were compared by using plates coated with various concentration of C277-ColV according to the following procedures. The plates were prepared according to the method described in Example 2 (9) using 0.1 pmol/cm^2 (0.1 μg/cm^2) - 416 pmol/cm^2 (20 μg/cm^2) of C277-ColV. 2 ml of a virus supernatant containing 1,000 cfu of PM5neo virus was added to respective plates and pre-incubation was carried out at 37 °C for 30 minutes, followed by washing thoroughly with PBS. To this plate was added 2 ml of DMEM medium containing 2,000 NIH/3T3 cells and the plate was incubated at 37°C for 24 hours.

The non-adhered cells were collected by decantation and the cells adhered to the plate were collected by trypsin treatment to detach them from the plate and these cells were combined. The resulting cell suspension was divided into two halves and one half portion was cultured in DMEM and the other portion was incubated in DMEM containing G418 at the final concentration of 0.75 mg/ml at 37°C for 10 days and the number of the colonies appeared was counted. A ratio of the number of $G418^r$ colonies relative to that of colonies obtained in a medium containing no G418 was taken as the gene transfer efficiency. The results are shown in Fig. 13. In Fig. 13, the abscissa indicates the functional material used and the ordinate indicates the gene transfer efficiency.

As shown in Fig. 13, when C277-ColV immobilized plate was used, the gene transfer efficiency was increased depending upon the concentration of C277-ColV used for immobilization.

(6) Gene transfer using polylysine

Binding of a polylysine [$(Lys)_n$] to a retrovirus was investigated by the following procedures. As a polylysine, poly-L-lysyine hydrobromide (molecular weight: 50,000-100,000, Wako Pure Chemical) was used and according to the same manner as described in Example 2 (9), it was immobilized on a plate by using 133 pmol/cm^2 (10 μg/cm^2) polylysine solution in PBS. The gene transfer efficiencies of this plate and a control plate on which BSA was immobilized was assessed according to the same manner as described in Example 4 (2). The results are shown in Fig. 14. In Fig. 14,

rately, as a control, a plate without CH-296, and a plate on which 32 pmol/cm^2 (2 μg/cm^2) or 127 pmol/cm^2 (8 μg/cm^2) of CH-296 was immobilized were prepared and the above procedures were carried out by adding a virus supernatant and cells thereto. The number of G418^r colonies thus obtained was counted and the results are summarized in Table 1.

Table 1

Plate	CH-296	Number of G418^r colonies
BSA	-	5
BSA	10 μg/ml	41
BSA	40 μg/ml	66
BSA	250 μg/ml	92
CH-296 (32 pmol/cm^2)	-	55
CH-296 (127 pmol/cm^2)	-	47

As shown in Table 1, when cell, virus and CH-296 were present together in the solution, the number of G418^r colonies was considerably increased in comparison with the absence of CH-296. The number was equal to or higher than that obtained by the use of the plate coated with CH-296. In addition, when a CH-296 solution was added, at the above respective concentrations, to a plate coated with BSA and, after allowing to stand for a while, the plate was washed and used for virus infection experiment, the number of G418^r colonies obtained was similar to that in the case without addition of CH-296 was obtained. From this, it is understood that CH-296 does not bind to a BSA immobilized. Therefore, it is considered that the above retrovirus infection promoting effect by CH-296 is not due to the adhesion of CH-296 in the solution to a plate during incubation.

(2) Gene transfer using functional material without immobilization

The effect on the retrovirus infection efficiency when polypeptides were present together on a plate without immobilization was investigated as follows. Namely, to a plate pre-coated with BSA according to the method described in Example 2 (9) was added each 2 ml of DMEM medium containing 1,000 cfu of PM5neo virus, 2,000 cells of NIH/3T3 cell, and C-FGF • A, ColV and C277-ColV at the final concentration of 1.67 nmol/ml, respectively, followed by incubation at 37 °C for 24 hours. The non-adhered cells were collected by decantation and the cells adhered to the plate were collected by trypsin treatment to remove them from the plate. These cells were combined. The resulting cell suspension was divided into two halves, one half portion was cultured with DMEM and the other portion was cultured with DMEM containing G418 at the final concentration of 0.75 mg/ml. Both portions were incubated at 37 °C for 10 days and the number of colonies which appeared was counted. A ratio of the number of G418^r colonies relative to that of colonies obtained on a medium containing no G418 was taken as the gene transfer efficiency. The results are shown in Fig. 18. In Fig. 18, the abscissa indicates the functional materials used and the ordinate indicates the gene transfer efficiency.

As shown in Fig. 18, when virus infection is taken place in the presence of each polypeptide, the higher gene transfer efficiency is obtained. Thus, it is clear that, even when these polypeptides are not immobilized on plates, the retrovirus infection is promoted.

(3) Gene transduction of non-adherent cells by using functional material without immobilization

The effect on the gene transfer efficiency into non-adherent cells by a polypeptide without immobilization was investigated as follows. Namely, to each of a plate prepared with 333 pmol/cm^2 (10 μg/cm^2) of H-271 and according to the same manner as that described in Example 2 (9) and a control plate on which BSA was immobilized was added 2 ml of RPMI medium (containing 5 ng/ml of GM-CFS, 50 units/ml of penicillin and 50 μg/ml of streptomycin) containing 1 X 10^4 cfu of TKNEO virus and 1 x 10^4 cells of TF-1 cells. To the BSA immobilized plate was further added H-271 at the final concentration of 50 μg/ml (1.67 nmol/ml) of H-271. Each plate was incubated at 37 °C for 24 hours. After incubation, the non-adhered cells were collected by decantation and the cells adhered to the plate were collected by trypsin treatment. These cells were combined. Each 1/5 portion of the resulting cell suspension was transferred to two plates coated with CH-296, incubated for 24 hours. The medium of one plate was exchanged with the above medium and the medium of the other plate was exchanged with the above medium containing G418 at the final concentration of 0.75 mg/ml. After incubation at 37°C for 8 days, the number of colonies which appeared was counted. The incidence (gene

plate.

Table 2

Beads	Number of G418^r colonies
BSA immobilized (control)	0
CH-296 immobilized	264

(2) Gene transfer into mouse bone marrow cells using beads on which functional material was immobilized

The possibility of increase in the retrovirus infection efficiency of mouse bone marrow cells with beads coated with the functional material was investigated according to the following procedures.

The mouse bone marrow cells were prepared according to the same manner as described in Example 4 (4) and pre-stimulated.

Each 2 ml of the medium, used for the above pre-stimulation, containing 1 X 10^6 pre-stimulated cells and 1 X 10^4 cfu of PM5neo virus was added to a plate coated with BSA according to the same manner as described in Example 2 (9) and the similar plate coated with BSA to which 1/10 portion of the CH-296 immobilized beads as prepared in Example 7 (1), followed by incubation at 37°C. After 2 hours, a medium (2ml) containing the same amount of virus was freshly added to each plate, followed by continuing incubation for 22 hours. After completion of incubation, the non-adhered cells were collected by decantation and the cells adhered to the plate were collected using a cell dissociation buffer (CDB, containing no enzymes, Gibco) and these cells were combined and washed twice with the same buffer. The number of the cells was counted. The collected cells were subjected to HPP-CFC assay according to the same manner as described in Example 4 (4).

The results are shown in Fig. 22. In Fig. 22, the abscissa indicates the functional material and its form used and the ordinate indicates the gene transfer efficiency. As shown in the results, it is understood that the retrovirus infection efficiency of mouse bone marrow cells can also be increase by using CH-296 immobilized beads.

Example 8

(1) Gene transfer using H-271 and CH-271

The effects of H-271 on retrovirus infection was assessed by pre-incubating a virus supernatant in plates coated with H-271 and CH-271 which was known to promote retrovirus infection, respectively, after thoroughly washing the plates, determining the remaining amount of the virus by NIH/3T3 cell colony formation assay and comparing the results of both plates. Namely, according to the same manner as described in Example 2 (9), plates were prepared with various concentrations of H-271 [67 pmol/cm^2 (2 μg/cm^2) to 333 pmol/cm^2 (10 μg/cm^2)] and CH-271 [67 pmol/cm^2 (4 μg/cm^2) to 333 pmol/cm^2 (20 μg/cm^2)], respectively. To each plate was added 2 ml of a virus supernatant containing 1,000 cfu of PM5neo virus and pre-incubated at 37°C for 30 minutes, followed by thoroughly washing with PBS. To this plate was added 2 ml of DMEM medium containing 2,000 NIH/3T3 cells and incubated at 37°C for 24 hours, followed by incubation in a selection medium containing 0.75 mg/ml of G418 for 10 days. Colonies were stained and counted. The results are shown in Fig. 23. Fig. 23 is a graph illustrating the relation between the functional material and the gene transfer efficiency. In Fig. 23, the abscissa indicates the amount of the functional material used and the ordinate indicates the number of G418^r colonies.

As shown in Fig. 23, when using CH-271 immobilized plate, the number of G418^r colonies appeared was almost the same regardless of the concentration of the polypeptide. On the other hand, in case of H-271, the number of colonies appeared was increased depending upon the concentration as increase in the concentration of the polypeptide used in immobilization and, in case of the plate prepared with 333 pmol/cm^2 of H-271, the number of the colonies appeared was almost the same as that of CH-271. This suggests that the equivalent virus infection efficiency to that of CH-271 can be obtained, when a sufficient amount of H-271 is immobilized on a plate.

(2) Gene transfer using C-FGF • A

The effects of C-FGF • A on retrovirus infection was investigated by NIH/3T3 cell colony assay. Namely, assessment was carried out according to the same manner as described in Example 8 (1) except for the use of plates prepared with 127 pmol/cm^2 (6 μg/cm^2) of C-FGF • A, 127 pmol/cm^2 (7.6 μg/cm^2) of CH-271 and 127 pmol/cm^2 (8 μg/cm^2) of CH-

tin.

Fig. 6 is a graph illustrating the gene transfer efficiencies into the target cells with the erythropoietin derivative, the polylysine and the mixture of the erythropoietin derivative and the polylysine.

Fig. 7 is a graph illustrating the gene transfer efficiencies into the target cells with the erythropoietin derivative, the fibronectin fragment polymer and the mixture of the erythropoietin derivative and the fibronectin fragment polymer.

Fig. 8 is a graph illustrating the gene transfer efficiencies into the target cells with the beads on which the fibronectin fragment was immobilized, the beads on which the cell binding domain polypeptide of fibronectin was immobilized and the beads on which the mixture of the fibronectin fragment and the cell binding domain polypeptide of fibronectin was immobilized.

Fig. 9 is a graph illustrating the transformation of the target cells with the fibroblast growth factor and the functional material containing the fibroblast growth factor.

Fig. 10 is a graph illustrating the relation between the amount of the functional material containing the fibroblast growth factor used and the gene transfer efficiency.

Fig. 11 is a graph illustrating the transformation of the target cells with the functional material containing fibroblast growth factor.

Fig. 12 is another graph illustrating the transformation of the target cells with the functional material containing the fibroblast growth factor.

Fig. 13 is a graph illustrating the relation between gene transfer efficiency into the target cells and the amount of the functional material containing the collagen fragment used.

Fig. 14 is a graph illustrating the gene transfer efficiency into the target cells with the polylysine.

Fig. 15 is a graph illustrating the transformation of the target cells with the fibronectin fragment and the fibronectin fragment polymer.

Fig. 16 is another graph illustrating the transformation of target cells with the fibronectin fragment and the fibronectin fragment polymer.

Fig. 17 is yet another graph illustrating the gene transfer efficiency into the target cells with the fibronectin fragment and the fibronectin fragment polymer.

Fig. 18 is a graph illustrating the gene transfer efficiency into the target cells with the functional material containing the fibroblast growth factor, the collagen fragment and the functional material containing the collagen fragment.

Fig. 19 is a graph illustrating the gene transfer efficiency into the target cells with the fibronectin fragment.

Fig. 20 is a graph illustrating the gene transfer efficiency into the target cells with the functional material containing the fibronectin fragment and fibroblast growth factor.

Fig. 21 is a graph illustrating the gene transfer efficiency into the target cells with the functional material containing the fibroblast growth factor and the fibronectin fragment.

Fig. 22 is a graph illustrating the gene transfer efficiency into the target cells with the fibronectin fragment immobilized beads.

Fig. 23 is a graph illustrating the relation between the amount of the fibronectin fragment used and the gene transduction of the target cells.

Fig. 24 is a graph illustrating the gene transduction of the target cells with the functional material containing the fibroblast growth factor and the fibronectin fragment.

Fig. 25 is another graph illustrating the gene transduction of the target cells with the functional material containing the fibroblast growth factor and the fibronectin fragment.

Fig. 26 is a graph illustrating the gene transduction of the target cells with the functional material containing the collagen fragment.

Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr
125 130 135
Pro Ile Gln Arg Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile
140 145 150
Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr
155 160 165
Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser
170 175 180
Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr
185 190 195
Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile
200 205 210
Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro Arg
215 220 225
Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile
230 235 240
Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala
245 250 255
Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys
260 265 270
Thr

SEQ. ID No. 2

LENGTH: 25

TYPE: amino acid

65 70 75

Gly Val Cys Ala Asn Arg Tyr Leu Ala Met Lys Glu Asp Gly Arg

80 85 90

Leu Leu Ala Ser Lys Cys Val Thr Asp Glu Cys Phe Phe Phe Glu

95 100 105

Arg Leu Glu Ser Asn Asn Tyr Asn Thr Tyr Arg Ser Arg Lys Tyr

110 115 120

Thr Ser Trp Tyr Val Ala Leu Lys Arg Thr Gly Gln Tyr Lys Leu

125 130 135

Gly Ser Lys Thr Gly Pro Gly Gln Lys Ala Ile Leu Phe Leu Pro

140 145 150

Met Ser Ala Lys Ser

155

SEQ. ID No. 4

LENGTH: 432

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg

1 5 10 15

Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu

20 25 30

215 220 225

Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys

230 235 240

Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg

245 250 255

Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg

260 265 270

Thr Glu Ile Asp Lys Pro Ser Met Ala Ala Gly Ser Ile Thr Thr

275 280 285

Leu Pro Ala Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro

290 295 300

Gly His Phe Lys Asp Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly

305 310 315

Phe Phe Leu Arg Ile His Pro Asp Gly Arg Val Asp Gly Val Arg

320 325 330

Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln Ala Glu Glu

335 340 345

Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu

350 355 360

Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys Val Thr

365 370 375

Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn

380 385 390

Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys

395 400 405

95 100 105

Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg

110 115 120

Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp

125 130 135

Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr

140 145 150

Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg

155 160 165

Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp

170 175 180

Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu

185 190 195

Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg

200 205 210

Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe

215 220 225

Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys

230 235 240

Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg

245 250 255

Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg

260 265 270

Thr Glu Ile Asp Lys Pro Ser Met Ala Ala Gly Ser Ile Thr Thr

275 280 285

SEQ. ID No. 6

LENGTH: 186

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

```
Gly Ile Arg Gly Leu Lys Gly Thr Lys Gly Glu Lys Gly Glu Asp
 1               5                  10                  15
Gly Phe Pro Gly Phe Lys Gly Asp Met Gly Ile Lys Gly Asp Arg
            20                  25                  30
Gly Glu Ile Gly Pro Pro Gly Pro Arg Gly Glu Asp Gly Pro Glu
            35                  40                  45
Gly Pro Lys Gly Arg Gly Gly Pro Asn Gly Asp Pro Gly Pro Leu
            50                  55                  60
Gly Pro Pro Gly Glu Lys Gly Lys Leu Gly Val Pro Gly Leu Pro
            65                  70                  75
Gly Tyr Pro Gly Arg Gln Gly Pro Lys Gly Ser Ile Gly Phe Pro
            80                  85                  90
Gly Phe Pro Gly Ala Asn Gly Glu Lys Gly Gly Arg Gly Thr Pro
            95                 100                 105
Gly Lys Pro Gly Pro Arg Gly Gln Arg Gly Pro Thr Gly Pro Arg
           110                 115                 120
Gly Glu Arg Gly Pro Arg Gly Ile Thr Gly Lys Pro Gly Pro Lys
           125                 130                 135
```

```
                    65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                    80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                    95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                   110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                   125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                   140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                   155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                   170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                   185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                   200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                   215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                   230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                   245                 250                 255
```

440 445 450

Lys Asp Gly Leu Pro Gly His Pro Gly Gln Arg Gly Glu Thr
455 460

SEQ. ID No. 8

LENGTH: 489

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
1 5 10 15

Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
20 25 30

Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
35 40 45

Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
50 55 60

Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
65 70 75

His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
80 85 90

Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
95 100 105

290 295 300

Asp Met Gly Ile Lys Gly Asp Arg Gly Glu Ile Gly Pro Pro Gly

305 310 315

Pro Arg Gly Glu Asp Gly Pro Glu Gly Pro Lys Gly Arg Gly Gly

320 325 330

Pro Asn Gly Asp Pro Gly Pro Leu Gly Pro Pro Gly Glu Lys Gly

335 340 345

Lys Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gly

350 355 360

Pro Lys Gly Ser Ile Gly Phe Pro Gly Phe Pro Gly Ala Asn Gly

365 370 375

Glu Lys Gly Gly Arg Gly Thr Pro Gly Lys Pro Gly Pro Arg Gly

380 385 390

Gln Arg Gly Pro Thr Gly Pro Arg Gly Glu Arg Gly Pro Arg Gly

395 400 405

Ile Thr Gly Lys Pro Gly Pro Lys Gly Asn Ser Gly Gly Asp Gly

410 415 420

Pro Ala Gly Pro Pro Gly Glu Arg Gly Pro Asn Gly Pro Gln Gly

425 430 435

Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly

440 445 450

Lys Asp Gly Leu Pro Gly His Pro Gly Gln Arg Gly Ala Ser Asp

455 460 465

Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His Gly

470 475 480

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CCATTAAAAT CAGCTAGCAG CAGACATTGG AAG 33

SEQ. ID No. 12

LENGTH: 36

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

TCTAGAGGAT CCTTAGCTAG CGCCTCTCTG TCCAGG 36

SEQ. ID No. 13

LENGTH: 547

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

Ala Ala Ser Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln
5 10 15

Val Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val
20 25 30

215 220 225

Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu

230 235 240

Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr

245 250 255

Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly

260 265 270

Arg Lys Lys Thr Ser Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe

275 280 285

Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro

290 295 300

Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu

305 310 315

Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser

320 325 330

Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys Tyr Glu Val

335 340 345

Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln

350 355 360

Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro Pro Arg Arg Ala

365 370 375

Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg

380 385 390

Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val Pro

395 400 405

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

Ala Ala Ser Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp
5 10 15

Thr Met Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr
20 25 30

Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val
35 40 45

Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr
50 55 60

Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val
65 70 75

Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr
80 85 90

Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala
95 100 105

Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr
110 115 120

Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro
125 130 135

Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr
140 145 150

Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu
155 160 165

```
                    350                 355                 360
Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn
                    365                 370                 375
Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr
                    380                 385                 390
Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly
                    395                 400                 405
Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln
                    410                 415                 420
Arg Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu
                    425                 430                 435
Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp
                    440                 445                 450
Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile
                    455                 460                 465
Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser
                    470                 475                 480
Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr
                    485                 490                 495
Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro Arg Glu Val Val
                    500                 505                 510
Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu
                    515                 520                 525
Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn
                    530                 535                 540
```

725 730 735

Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr Pro

740 745 750

Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile Thr

755 760 765

Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro Arg Glu

770 775 780

Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile Thr

785 790 795

Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala Leu

800 805 810

Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys Thr

815 820 825

Ser

SEQ. ID No. 15

LENGTH: 38

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

AAACCATGGC AGCTAGCGCT ATTCCTGCAC CAACTGAC 38

SEQ. ID No. 16

ATCGACGCCT CCACTGCCAT TGATGCACCA TCCAACCTGC GTTTCCTGGC CACCACACCC 600

AATTCCTTGC TGGTATCATG GCAGCCGCCA CGTGCCAGGA TTACCGGCTA CATCATCAAG 660

TATGAGAAGC CTGGGTCTCC TCCCAGAGAA GTGGTCCCTC GGCCCCGCCC TGGTGTCACA 720

GAGGCTACTA TTACTGGCCT GGAACCGGGA ACCGAATATA CAATTTATGT CATTGCCCTG 780

AAGAATAATC AGAAGAGCGA GCCCCTGATT GGAAGGAAAA AGACTAGCGC TATTCCTGCA 840

CCAACTGACC TGAAGTTCAC TCAGGTCACA CCCACAAGCC TGAGCGCCCA GTGGACACCA 900

CCCAATGTTC AGCTCACTGG ATATCGAGTG CGGGTGACCC CCAAGGAGAA GACCGGACCA 960

ATGAAAGAAA TCAACCTTGC TCCTGACAGC TCATCCGTGG TTGTATCAGG ACTTATGGTG 1020

GCCACCAAAT ATGAAGTGAG TGTCTATGCT CTTAAGGACA CTTTGACAAG CAGACCAGCT 1080

CAGGGTGTTG TCACCACTCT GGAGAATGTC AGCCCACCAA GAAGGGCTCG TGTGACAGAT 1140

GCTACTGAGA CCACCATCAC CATTAGCTGG AGAACCAAGA CTGAGACGAT CACTGGCTTC 1200

CAAGTTGATG CCGTTCCAGC CAATGGCCAG ACTCCAATCC AGAGAACCAT CAAGCCAGAT 1260

GTCAGAAGCT ACACCATCAC AGGTTTACAA CCAGGCACTG ACTACAAGAT CTACCTGTAC 1320

ACCTTGAATG ACAATGCTCG GAGCTCCCCT GTGGTCATCG ACGCCTCCAC TGCCATTGAT 1380

GCACCATCCA ACCTGCGTTT CCTGGCCACC ACACCCAATT CCTTGCTGGT ATCATGGCAG 1440

CCGCCACGTG CCAGGATTAC CGGCTACATC ATCAAGTATG AGAAGCCTGG GTCTCCTCCC 1500

AGAGAAGTGG TCCCTCGGCC CCGCCCTGGT GTCACAGAGG CTACTATTAC TGGCCTGGAA 1560

CCGGGAACCG AATATACAAT TTATGTCATT GCCCTGAAGA ATAATCAGAA GAGCGAGCCC 1620

CTGATTGGAA GGAAAAAGAC TAGT 1644

SEQ. ID No. 18

LENGTH: 37

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

```
TTTTCTGATA TTACTGCCAA CTCTTTTACT GTGCACTGGA TTGCTCCTCG AGCCACCATC  360
ACTGGCTACA GGATCCGCCA TCATCCCGAG CACTTCAGTG GGAGACCTCG AGAAGATCGG  420
GTGCCCCACT CTCGGAATTC CATCACCCTC ACCAACCTCA CTCCAGGCAC AGAGTATGTG  480
GTCAGCATCG TTGCTCTTAA TGGCAGAGAG GAAAGTCCCT TATTGATTGG CCAACAATCA  540
ACAGTTTCTG ATGTTCCGAG GGACCTGGAA GTTGTTGCTG CGACCCCCAC CAGCCTACTG  600
ATCAGCTGGG ATGCTCCTGC TGTCACAGTG AGATATTACA GGATCACTTA CGGAGAAACA  660
GGAGGAAATA GCCCTGTCCA GGAGTTCACT GTGCCTGGGA GCAAGTCTAC AGCTACCATC  720
AGCGGCCTTA AACCTGGAGT TGATTATACC ATCACTGTGT ATGCTGTCAC TGGCCGTGGA  780
GACAGCCCCG CAAGCAGCAA GCCAATTTCC ATTAATTACC GAACAGAAAT TGACAAACCA  840
TCCACTAGCG CTATTCCTGC ACCAACTGAC CTGAAGTTCA CTCAGGTCAC ACCCACAAGC  900
CTGAGCGCCC AGTGGACACC ACCCAATGTT CAGCTCACTG GATATCGAGT GCGGGTGACC  960
CCCAAGGAGA AGACCGGACC AATGAAAGAA ATCAACCTTG CTCCTGACAG CTCATCCGTG 1020
GTTGTATCAG GACTTATGGT GGCCACCAAA TATGAAGTGA GTGTCTATGC TCTTAAGGAC 1080
ACTTTGACAA GCAGACCAGC TCAGGGTGTT GTCACCACTC TGGAGAATGT CAGCCCACCA 1140
AGAAGGGCTC GTGTGACAGA TGCTACTGAG ACCACCATCA CCATTAGCTG GAGAACCAAG 1200
ACTGAGACGA TCACTGGCTT CCAAGTTGAT GCCGTTCCAG CCAATGGCCA GACTCCAATC 1260
CAGAGAACCA TCAAGCCAGA TGTCAGAAGC TACACCATCA CAGGTTTACA ACCAGGCACT 1320
GACTACAAGA TCTACCTGTA CACCTTGAAT GACAATGCTC GGAGCTCCCC TGTGGTCATC 1380
GACGCCTCCA CTGCCATTGA TGCACCATCC AACCTGCGTT TCCTGGCCAC CACACCCAAT 1440
TCCTTGCTGG TATCATGGCA GCCGCCACGT GCCAGGATTA CCGGCTACAT CATCAAGTAT 1500
GAGAAGCCTG GGTCTCCTCC CAGAGAAGTG GTCCCTCGGC CCCGCCCTGG TGTCACAGAG 1560
GCTACTATTA CTGGCCTGGA ACCGGGAACC GAATATACAA TTTATGTCAT TGCCCTGAAG 1620
AATAATCAGA AGAGCGAGCC CCTGATTGGA AGGAAAAAGA CTAGCGCTAT TCCTGCACCA 1680
ACTGACCTGA AGTTCACTCA GGTCACACCC ACAAGCCTGA GCGCCCAGTG GACACCACCC 1740
AATGTTCAGC TCACTGGATA TCGAGTGCGG GTGACCCCCA AGGAGAAGAC CGGACCAATG 1800
```

35 40 45

Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu

50 55 60

Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln

65 70 75

His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp

80 85 90

Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe

95 100 105

Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg

110 115 120

Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp

125 130 135

Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr

140 145 150

Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg

155 160 165

Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp

170 175 180

Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu

185 190 195

Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg

200 205 210

Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe

215 220 225

410 415 420

Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr

425 430 435

Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser

440 445 450

Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser

455 460 465

Asn Leu Arg Phe Leu Ala Thr

470

SEQ. ID No. 22

LENGTH: 457

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg

1 5 10 15

Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu

20 25 30

Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu

35 40 45

Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu

50 55 60

245 250 255

Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg

260 265 270

Thr Glu Ile Asp Lys Pro Ser Met Asn Val Ser Pro Pro Arg Arg

275 280 285

Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp

290 295 300

Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val

305 310 315

Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys Pro Asp

320 325 330

Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr

335 340 345

Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro

350 355 360

Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu

365 370 375

Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln

380 385 390

Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys

395 400 405

Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly

410 415 420

Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr

425 430 435

```
                95                  100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
            110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
        125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
    140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
            170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
        185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
    200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
            230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
        245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
    260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Ala Ile Pro Ala Pro Thr Asp
275                 280                 285
```

470 475 480

Trp Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr

485 490 495

Glu Lys Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg

500 505 510

Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr

515 520 525

Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser

530 535 540

Glu Pro Leu Ile Gly Arg Lys Lys Thr

545

SEQ. ID No. 24

LENGTH: 574

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg

1 5 10 15

Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu

20 25 30

Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu

35 40 45

230 235 240

Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg

245 250 255

Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg

260 265 270

Thr Glu Ile Asp Lys Pro Ser Met Ala Ile Pro Ala Pro Thr Asp

275 280 285

Leu Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp

290 295 300

Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr

305 310 315

Pro Lys Glu Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro

320 325 330

Asp Ser Ser Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys

335 340 345

Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg

350 355 360

Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro Pro

365 370 375

Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile

380 385 390

Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp

395 400 405

Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys

410 415 420

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
 1               5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
            20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
        35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
    50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
            80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
        95                  100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
    110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
            140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
```

```
ATGCCCACTG ACCTGCGATT CACCAACATT GGTCCAGACA CCATGCGTGT CACCTGGGCT   60
CCACCCCCAT CCATTGATTT AACCAACTTC CTGGTGCGTT ACTCACCTGT GAAAAATGAG  120
GAAGATGTTG CAGAGTTGTC AATTTCTCCT TCAGACAATG CAGTGGTCTT AACAAATCTC  180
CTGCCTGGTA CAGAATATGT AGTGAGTGTC TCCAGTGTCT ACGAACAACA TGAGAGCACA  240
CCTCTTAGAG GAAGACAGAA AACAGGTCTT GATTCCCCAA CTGGCATTGA CTTTTCTGAT  300
ATTACTGCCA ACTCTTTTAC TGTGCACTGG ATTGCTCCTC GAGCCACCAT CACTGGCTAC  360
AGGATCCGCC ATCATCCCGA GCACTTCAGT GGGAGACCTC GAGAAGATCG GGTGCCCCAC  420
TCTCGGAATT CCATCACCCT CACCAACCTC ACTCCAGGCA CAGAGTATGT GGTCAGCATC  480
GTTGCTCTTA ATGGCAGAGA GGAAAGTCCC TTATTGATTG GCCAACAATC AACAGTTTCT  540
GATGTTCCGA GGGACCTGGA AGTTGTTGCT GCGACCCCCA CCAGCCTACT GATCAGCTGG  600
GATGCTCCTG CTGTCACAGT GAGATATTAC AGGATCACTT ACGGAGAAAC AGGAGGAAAT  660
AGCCCTGTCC AGGAGTTCAC TGTGCCTGGG AGCAAGTCTA CAGCTACCAT CAGCGGCCTT  720
AAACCTGGAG TTGATTATAC CATCACTGTG TATGCTGTCA CTGGCCGTGG AGACAGCCCC  780
GCAAGCAGCA AGCCAATTTC CATTAATTAC CGAACAGAAA TTGACAAACC ATCCATGGCA  840
GCCGGGAGCA TCACCACGCT GCCCGCCTTG CCCGAGGATG GCGGCAGCGG CGCCTTCCCG  900
CCCGGCCACT TCAAGGACCC CAAGCGGCTG TACTGCAAAA ACGGGGGCTT CTTCCTGCGC  960
ATCCACCCCG ACGGCCGAGT TGACGGGGTC CGGGAGAAGA GCGACCCTCA CATCAAGCTA 1020
CAACTTCAAG CAGAAGAGAG AGGAGTTGTG TCTATCAAAG GAGTGTGTGC TAACCGTTAC 1080
CTGGCTATGA AGGAAGATGG AAGATTACTG GCTTCTAAAT GTGTTACGGA TGAGTGTTTC 1140
TTTTTTGAAC GATTGGAATC TAATAACTAC AATACTTACC GCTCAAGGAA ATACACCAGT 1200
TGGTATGTGG CACTGAAACG AACTGGGCAG TATAAACTTG GATCCAAAAC AGGACCTGGG 1260
CAGAAAGCTA TACTTTTTCT TCCAATGTCT GCTGCTAGCG ACGAGCTTCC CCAACTGGTA 1320
ACCCTTCCAC ACCCCAATCT TCATGGACCA GAGATCTTGG ATGTTCCTTC CACA         1374
```

SEQ. ID No. 27

CCAGCTCAGG GTGTTGTCAC CACTCTGGAG AATGTCAGCC CACCAAGAAG GGCTCGTGTG 1140

ACAGATGCTA CTGAGACCAC CATCACCATT AGCTGGAGAA CCAAGACTGA GACGATCACT 1200

GGCTTCCAAG TTGATGCCGT TCCAGCCAAT GGCCAGACTC CAATCCAGAG AACCATCAAG 1260

CCAGATGTCA GAAGCTACAC CATCACAGGT TTACAACCAG GCACTGACTA CAAGATCTAC 1320

CTGTACACCT TGAATGACAA TGCTCGGAGC TCCCCTGTGG TCATCGACGC CTCCACTGCC 1380

ATTGATGCAC CATCCAACCT GCGTTTCCTG GCCACC 1416

SEQ. ID No. 28

LENGTH: 35

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

Gly Gly Arg Gly Thr Pro Gly Lys Pro Gly Pro Arg Gly Gln Arg
1 5 10 15

Gly Pro Thr Gly Pro Arg Gly Glu Arg Gly Pro Arg Gly Ile Thr
20 25 30

Gly Lys Pro Gly Pro
35

SEQ. ID No. 29

LENGTH: 302

TYPE: amino acid

STRANDEDNESS: single

Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
170 175 180

Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
185 190 195

Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
200 205 210

Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
215 220 225

Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
230 235 240

Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
245 250 255

Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
260 265 270

Thr Glu Ile Asp Lys Pro Ser Asp Glu Leu Pro Gln Leu Val Thr
275 280 285

Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro
290 295 300

Ser Thr

SEQ. ID No. 30

LENGTH: 573

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

```
                    170                 175                 180
Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe
                    185                 190                 195
Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro
                    200                 205                 210
Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly
                    215                 220                 225
Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr
                    230                 235                 240
Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile
                    245                 250                 255
Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile
                    260                 265                 270
Gly Arg Lys Lys Thr Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe
                    275                 280                 285
Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro
                    290                 295                 300
Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu
                    305                 310                 315
Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser
                    320                 325                 330
Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys Tyr Glu Val
                    335                 340                 345
Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln
                    350                 355                 360
```

545 550 555

Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro

560 565 570

Ser Thr Ser

SEQ. ID No. 31

LENGTH: 37

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

AAACCATGGC AGCTAGCAAT GTCAGCCCAC CAAGAAG 37

SEQ. ID No. 32

LENGTH: 37

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

AAAGGATCCC TAACTAGTGG AAGGAACATC CAAGATC 37

SEQ. ID No. 33

LENGTH: 1722

GCTACTGAGA CCACCATCAC CATTAGCTGG AGAACCAAGA CTGAGACGAT CACTGGCTTC 1200

CAAGTTGATG CCGTTCCAGC CAATGGCCAG ACTCCAATCC AGAGAACCAT CAAGCCAGAT 1260

GTCAGAAGCT ACACCATCAC AGGTTTACAA CCAGGCACTG ACTACAAGAT CTACCTGTAC 1320

ACCTTGAATG ACAATGCTCG GAGCTCCCCT GTGGTCATCG ACGCCTCCAC TGCCATTGAT 1380

GCACCATCCA ACCTGCGTTT CCTGGCCACC ACACCCAATT CCTTGCTGGT ATCATGGCAG 1440

CCGCCACGTG CCAGGATTAC CGGCTACATC ATCAAGTATG AGAAGCCTGG GTCTCCTCCC 1500

AGAGAAGTGG TCCCTCGGCC CCGCCCTGGT GTCACAGAGG CTACTATTAC TGGCCTGGAA 1560

CCGGGAACCG AATATACAAT TTATGTCATT GCCCTGAAGA ATAATCAGAA GAGCGAGCCC 1620

CTGATTGGAA GGAAAAAGAC TAGCGACGAG CTTCCCCAAC TGGTAACCCT TCCACACCCC 1680

AATCTTCATG GACCAGAGAT CTTGGATGTT CCTTCCACTA GT 1722

SEQ. ID No. 34

LENGTH: 412

TYPE: amino acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: peptide

SEQUENCE:

Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln
5 10 15

Pro Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu
20 25 30

His Leu Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys
35 40 45

Phe Glu Leu Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp

Ser Arg Val Leu Gln Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu
245 250 255
Asn Ile Thr Thr Gly Cys Ala Glu His Cys Ser Leu Asn Glu Asn
260 265 270
Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg
275 280 285
Met Glu Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala
290 295 300
Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu Leu Val Asn
305 310 315
Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp Lys Ala
320 325 330
Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu Gly
335 340 345
Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
350 355 360
Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg
365 370 375
Val Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly
380 385 390
Glu Ala Cys Arg Thr Gly Asp Arg Leu Ala Met Asp Pro Leu Glu
395 400 405
Ser Thr Arg Ala Ala Ala Ser
410

GGCCTCCCGA ATTCCGGTGC CCCACCACGC CTC 33

SEQ. ID No. 38

LENGTH: 33

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (synthetic DNA)

SEQUENCE:

CCCACGTGGA TCCATGGCTA ATCTGTCCCC TGT 33

SEQ. ID No. 39

LENGTH: 1239

TYPE: nucleic acid

STRANDEDNESS: single

TOPOLOGY: linear

MOLECULAR TYPE: other nucleic acid (DNA encoding an artificial polypeptide)

SEQUENCE:

ATGTCCCCTA TACTAGGTTA TTGGAAAATT AAGGGCCTTG TGCAACCCAC TCGACTTCTT 60

TTGGAATATC TTGAAGAAAA ATATGAAGAG CATTTGTATG AGCGCGATGA AGGTGATAAA 120

TGGCGAAACA AAAAGTTTGA ATTGGGTTTG GAGTTTCCCA ATCTTCCTTA TTATATTGAT 180

GGTGATGTTA AATTAACACA GTCTATGGCC ATCATACGTT ATATAGCTGA CAAGCACAAC 240

ATGTTGGGTG GTTGTCCAAA AGAGCGTGCA GAGATTTCAA TGCTTGAAGG AGCGGTTTTG 300

GATATTAGAT ACGGTGTTTC GAGAATTGCA TATAGTAAAG ACTTTGAAAC TCTCAAAGTT 360

a mixture of an effective amount of a functional material having retrovirus binding domain, and an effective amount of another functional material having target cell binding domain.

10. A culture medium according to claim 9, wherein the functional material having retrovirus binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

11. A culture medium according to claim 9, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

12. A culture medium according to claim 11, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

13. A culture medium according to claim 12, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

14. A culture medium according to claim 13, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

15. A culture medium according to claim 12, wherein the ligand is erythropoietin.

16. A culture medium according to any one of claims 9 to 15, wherein the functional materials are immobilized.

17. A method for localization of a retrovirus which comprises incubating a culture medium containing the retrovirus contacted with a mixture of an effective amount of a functional material having retrovirus binding domain, and an effective amount of another functional material having target cell binding domain.

18. A method for localization according to claim 17, wherein the functional material having retrovirus binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

19. A method for localization according to claim 17, wherein the functional material having target cell binding domain is a ligand which specifically binds to the target cells.

20. A method for localization according to claim 19, wherein the ligand is selected from cell adhesion proteins, hormones, cytokines, antibodies, sugar chains, carbohydrates and metabolites.

21. A method for localization according to claim 20, wherein the cell adhesion protein is a cell binding domain polypeptide of fibronectin.

22. A method for localization according to claim 21, wherein the cell binding domain polypeptide of fibronectin is a polypeptide of the binding domain to VLA-5 and/or VLA-4.

23. A method for localization according to claim 20, wherein the ligand is erythropoietin.

24. A method for localization according to any one of claims 17 to 23, wherein the functional materials are immobilized.

25. A kit for carrying out retrovirus-mediated gene transfer into target cells, which comprises:

(a) an effective amount of a functional material having retrovirus binding domain and/or an effective amount of another functional material having target cell binding domain;
(b) an artificial substrate for incubating the retrovirus and the target cells; and
(c) a target cell growth factor for pre-stimulating the target cells.

26. A kit according to claim 25, wherein the functional material having retrovirus binding domain is a functional material selected from Heparin-II binding region of fibronectin, fibroblast growth factors, collagens, polylysines and functional equivalents thereof.

47. A method according to any one of claims 35 to 45, wherein the functional material is used without immobilization.

48. A culture medium for target cells to be used for gene transfer into the target cells with a retrovirus which comprises an effective amount of a functional material having a target cell binding domain, and a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

49. A culture medium according to claim 48, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalent of the factor.

50. A culture medium according to claim 48, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

51. A culture medium according to claim 48, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalents of the fragment.

52. A culture medium according to claim 48, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

53. A culture medium according to claim 48, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

54. A culture medium according to any one of claims 48 to 53, wherein the functional material is immobilized.

55. A method for localization of a retrovirus which comprises incubating a culture medium containing the retrovirus contacted with an effective amount of a functional material having a target cell binding domain, and a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule.

56. A method for localization according to claim 55, wherein the fibroblast growth factor is selected from a fibroblast growth factor represented by SEQ. ID No. 3 of the Sequence Listing, functional equivalents of the factor and polypeptides containing the factor or functional equivalents of the factor.

57. A method for localization according to claim 55, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 4 or 5 of the Sequence Listing.

58. A method for localization according to claim 55, wherein the collagen is selected from a fragment having insulin binding domain derived from type V collagen, functional equivalents of the fragment and polypeptides containing the fragment or functional equivalent of the fragment.

59. A method for localization according to claim 58, wherein the fragment having insulin binding domain derived from type V collagen is a fragment having an amino acid sequence represented by SEQ. ID No. 6 of the Sequence Listing.

60. A method for localization according to claim 55, wherein the functional material is a polypeptide having an amino acid sequence represented by SEQ. ID No. 7 or 8 of the Sequence Listing.

61. A method for localization according to any one of claims 50 to 60, wherein the functional material is immobilized.

62. A kit for carrying out retrovirus-mediated gene transfer into target cells, which comprises:

(a) an effective amount of a functional material having a target cell binding domain, and a retrovirus binding domain derived from a fibroblast growth factor, a collagen or a polylysine, or a functional equivalent thereof on the same molecule;
(b) an artificial substrate for incubating the retrovirus and the target cells; and
(c) a target cell growth factor for pre-stimulating the target cells.

81. A polypeptide represented by SEQ. ID 13 of the Sequence Listing.

82. A gene encoding the polypeptide according to claim 81.

83. A gene according to claim 82 which is represented by SEQ. ID No. 17 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a retrovirus.

84. A polypeptide represented by SEQ. ID No. 30 of the Sequence Listing or functional equivalents thereof.

85. A gene encoding the polypeptide according to claim 84.

86. A gene according to claim 85 which is represented by SEQ. ID No. 33 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a retrovirus.

87. A polypeptide represented by SEQ. ID No. 5 of the Sequence Listing or functional equivalents thereof.

88. A gene encoding the polypeptide according to claim 87.

89. A gene according to claim 88 which is represented by SEQ. ID No. 26 of the Sequence Listing, or a gene hybridizable thereto under stringent conditions and encoding a polypeptide which improves the efficiency of gene transfer into target cells with a retrovirus.

Fig. 1

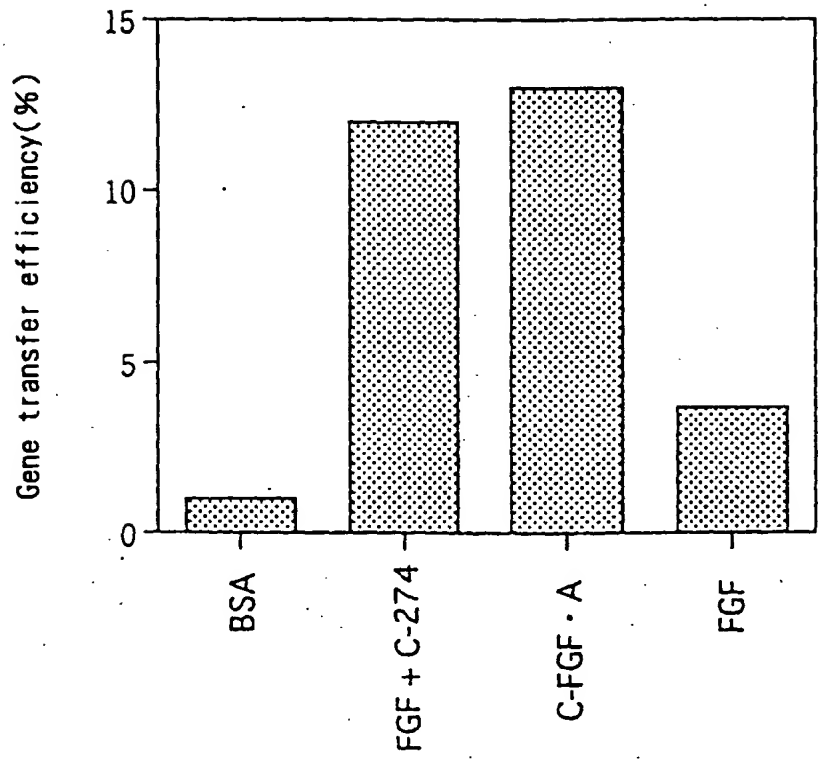


Fig. 2

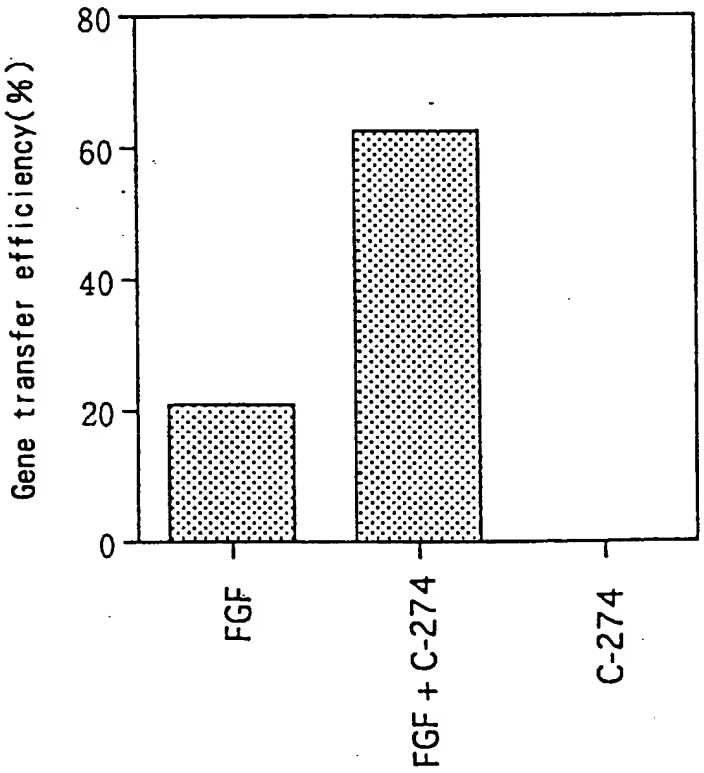


Fig. 3

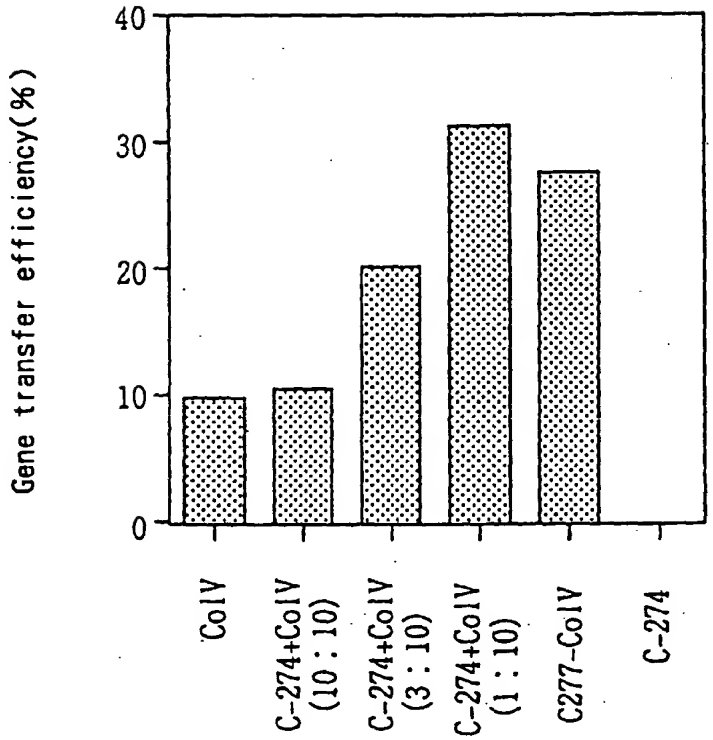

Gene transfer efficiency(%)
40
30
20
10
0
ColV
C-274+ColV (10 : 10)
C-274+ColV (3 : 10)
C-274+ColV (1 : 10)
C277-ColV
C-274

Fig. 4

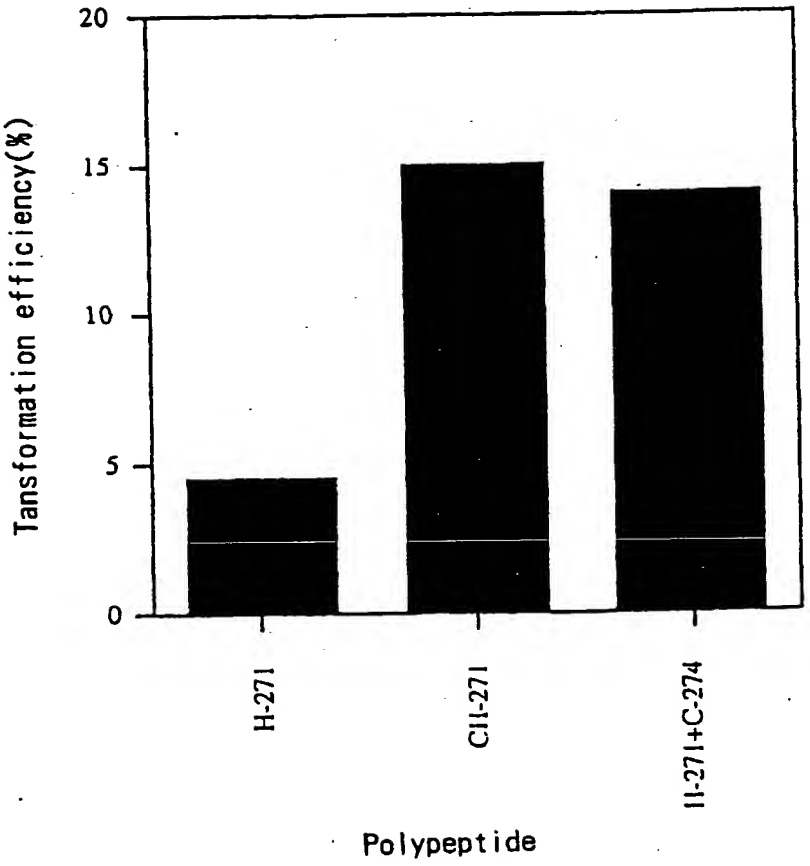

20
15
10
5
0
Tansformation efficiency(%)
H-271
CII-271
II-271+C-274
Polypeptide

Fig. 5

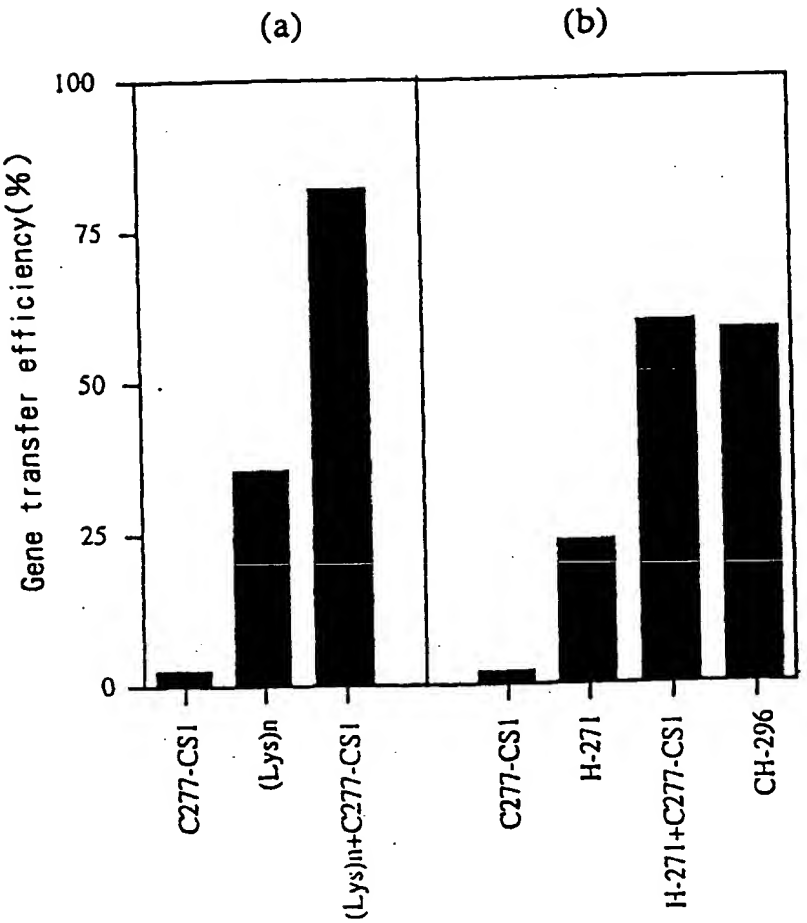

(a)
(b)
100
75
50
25
0
Gene transfer efficiency(%)
C277-CS1
(Lys)n
(Lys)n+C277-CS1
C277-CS1
H-271
H-271+C277-CS1
CH-296

Fig. 6

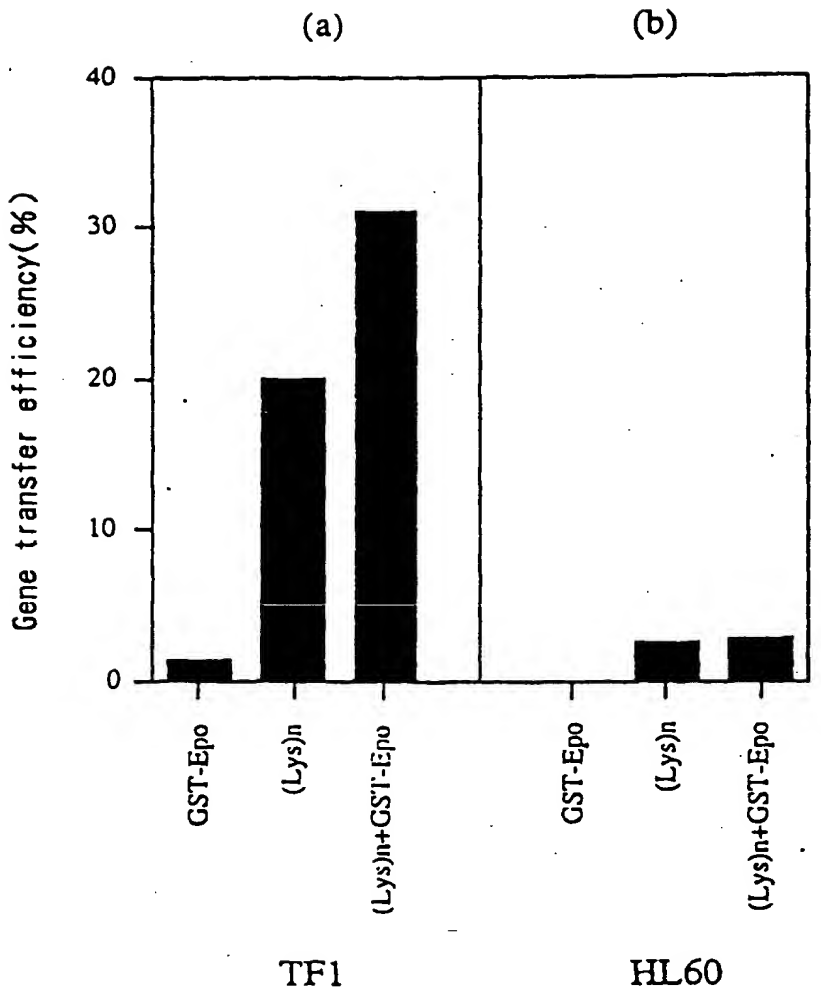

(a)
(b)
40
30
20
10
0
Gene transfer efficiency(%)
GST-Epo
(Lys)n
(Lys)n+GST-Epo
GST-Epo
(Lys)n
(Lys)n+GST-Epo
TF1
HL60

Fig. 7

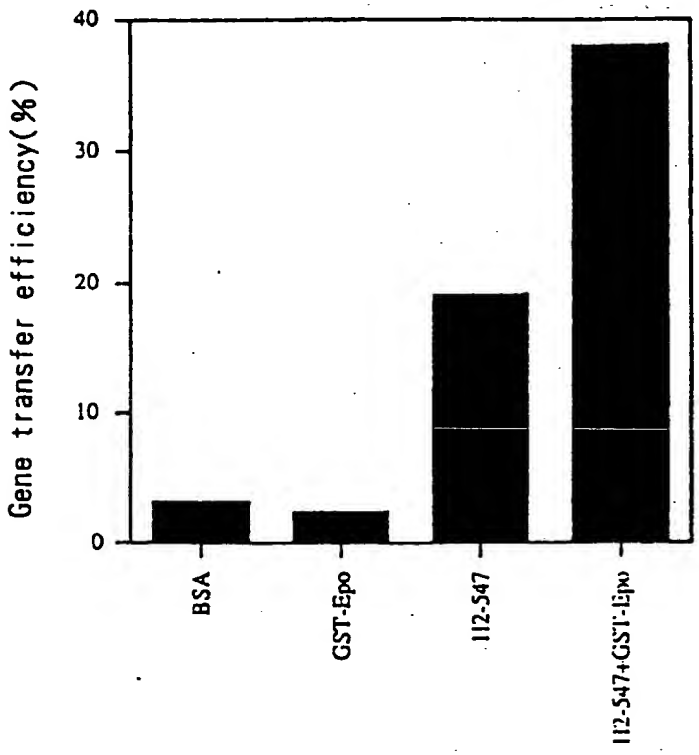

Gene transfer efficiency(%)
40
30
20
10
0
BSA
GST-Epo
112-547
112-547+GST-Epo

Fig. 8

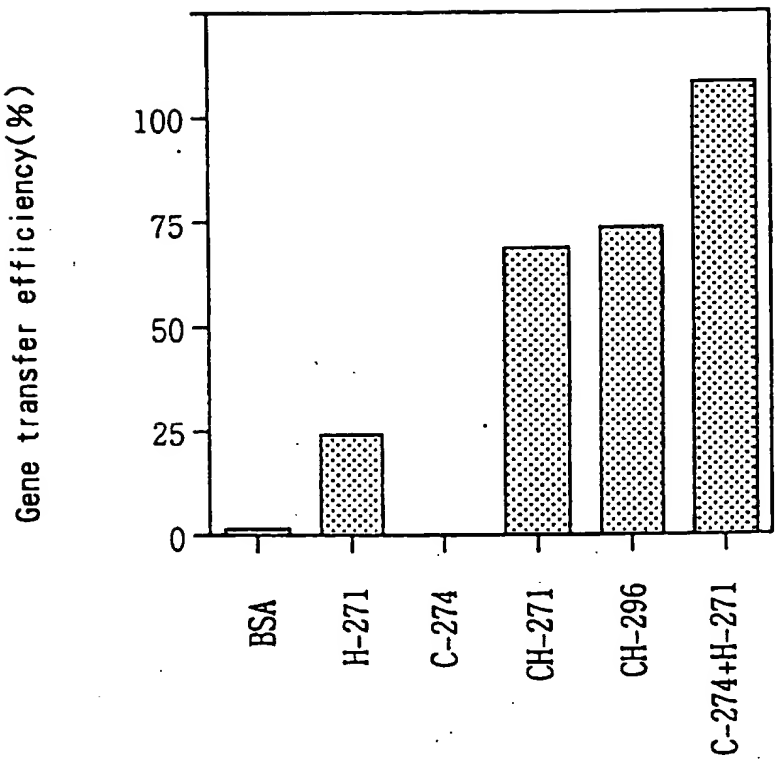

Gene transfer efficiency(%)
100
75
50
25
0
BSA
H-271
C-274
CH-271
CH-296
C-274+H-271

Fig. 9

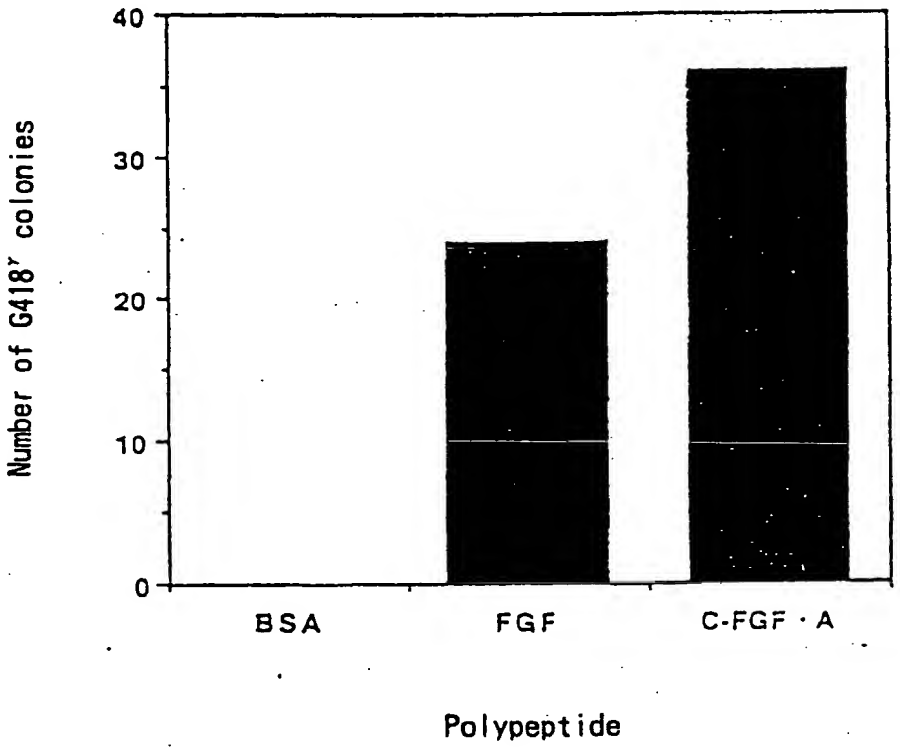

40
30
20
10
0
Number of G418^{r} colonies
BSA
FGF
C-FGF・A
Polypeptide

Fig. 10

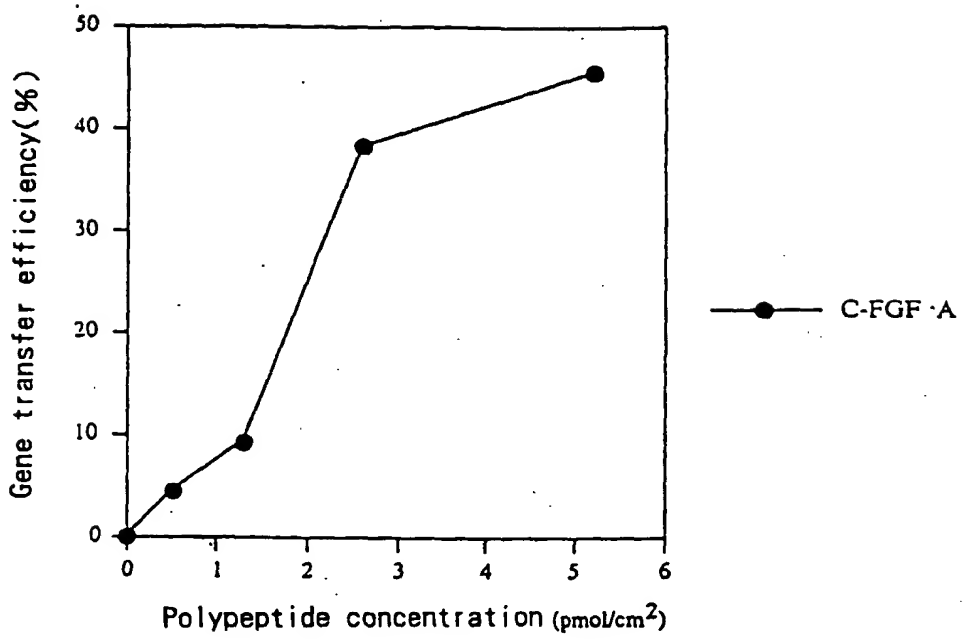

Gene transfer efficiency(%)
50
40
30
20
10
0
0
1
2
3
4
5
6
Polypeptide concentration (pmol/cm2)
C-FGF-A

Fig. 11

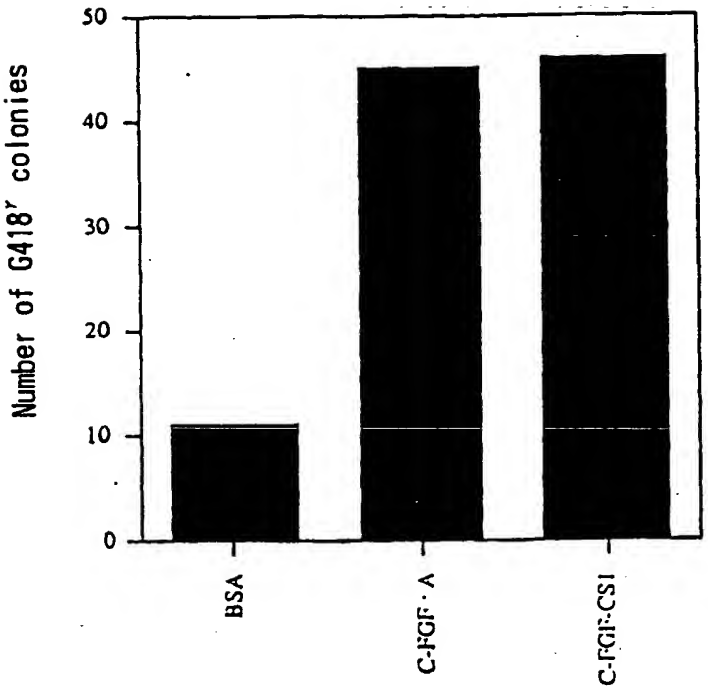


Fig. 12

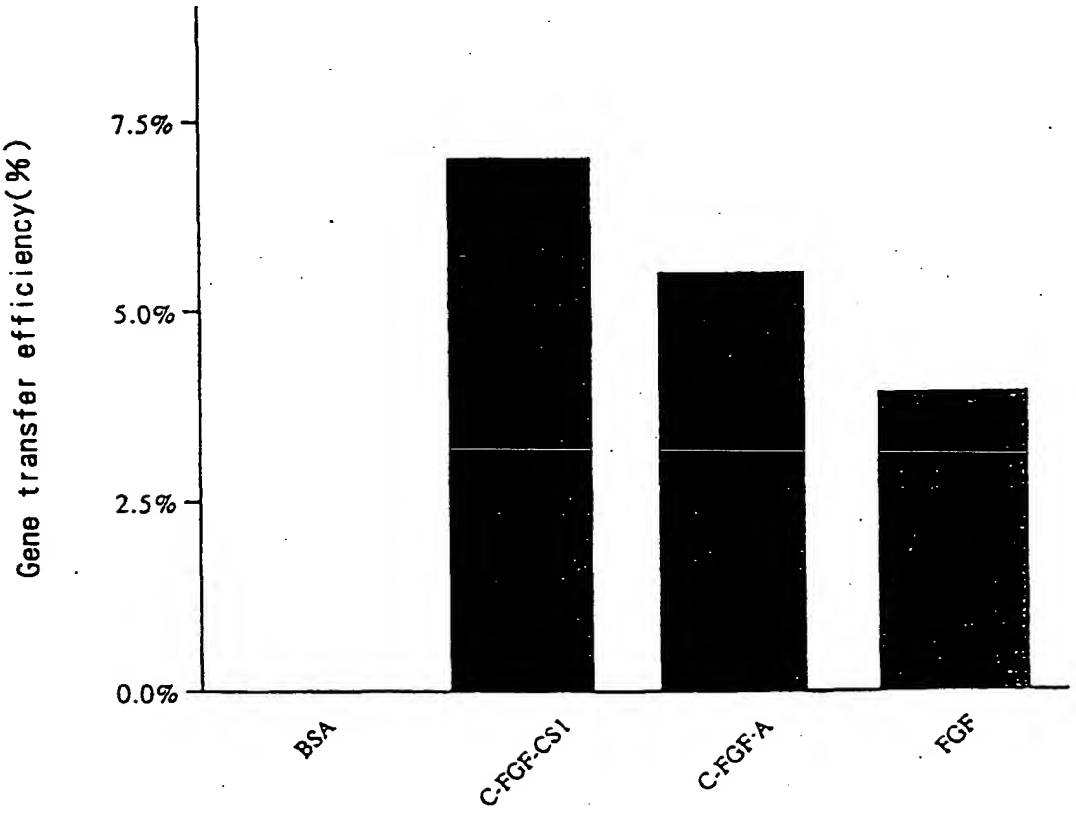


Fig. 13

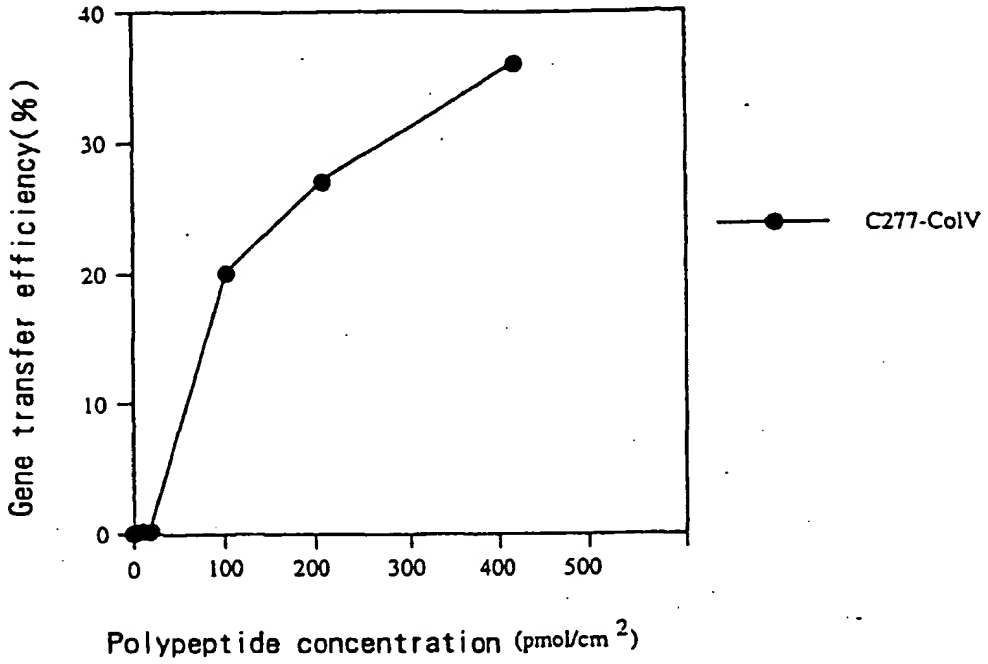

Gene transfer efficiency(%)
40
30
20
10
0
0
100
200
300
400
500
Polypeptide concentration (pmol/cm 2)
C277-ColV

Fig. 14

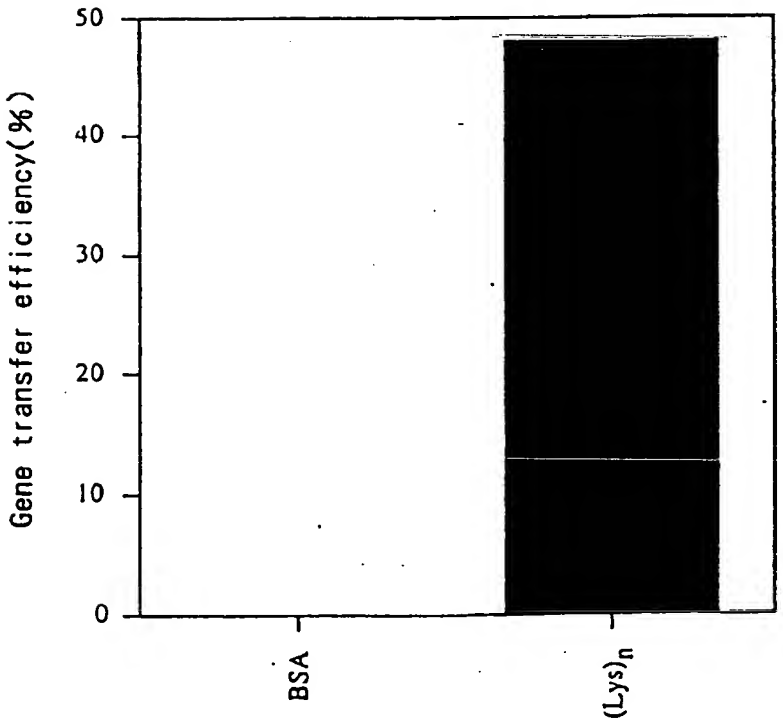


Fig. 15

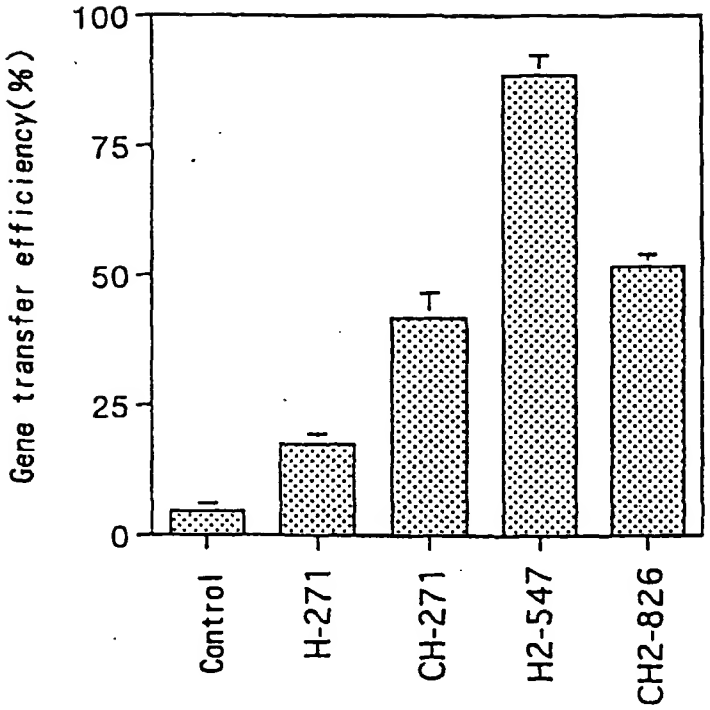

Gene transfer efficiency(%)
100
75
50
25
0
Control
H-271
CH-271
H2-547
CH2-826

Fig. 16

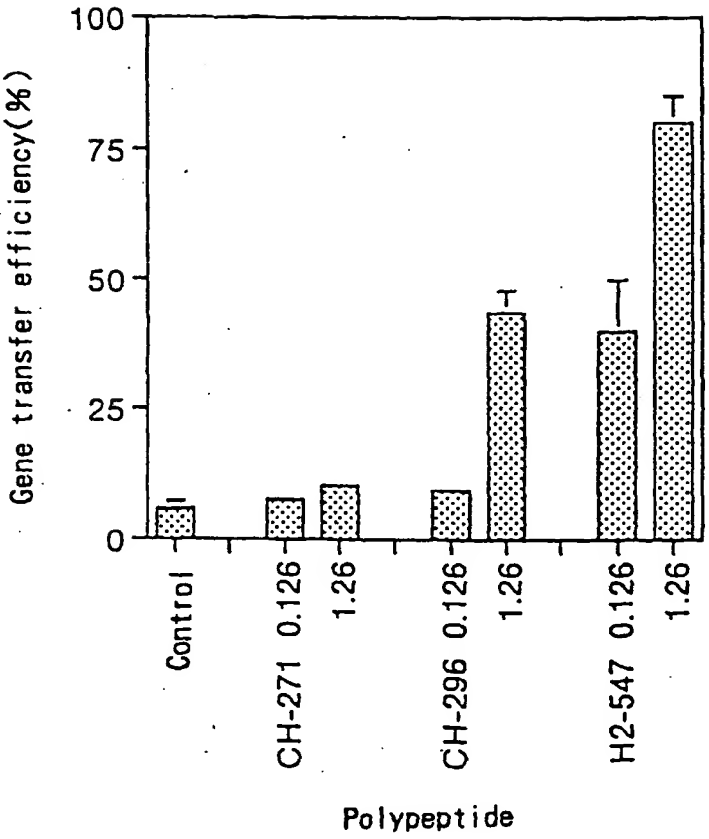

Gene transfer efficiency(%)
100
75
50
25
0
Control
CH-271 0.126
1.26
CH-296 0.126
1.26
H2-547 0.126
1.26
Polypeptide

Fig. 17

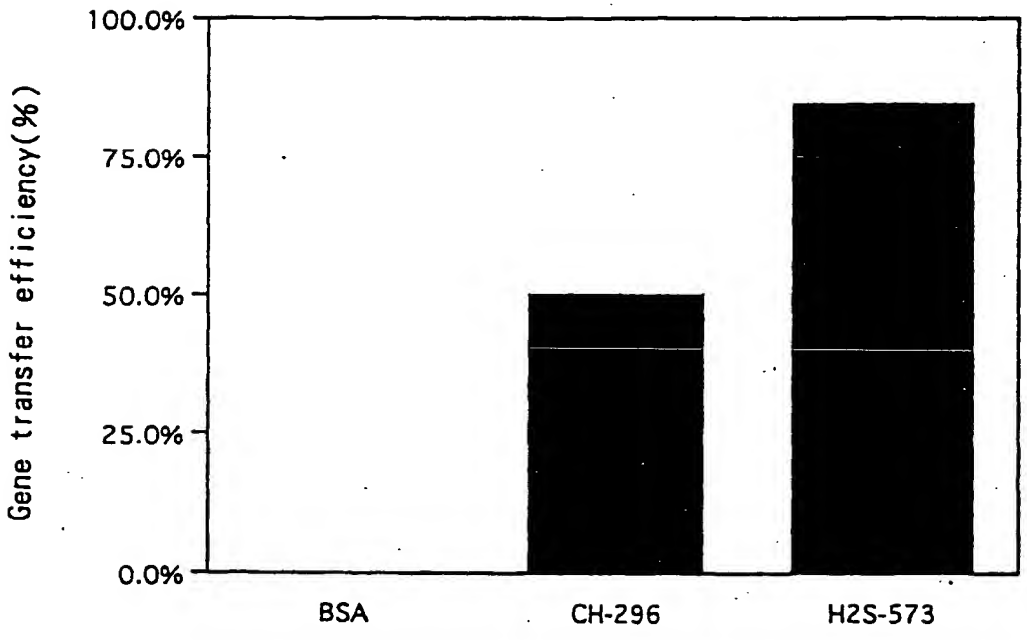

100.0%
75.0%
50.0%
25.0%
0.0%
Gene transfer efficiency(%)
BSA
CH-296
H2S-573

Fig. 18

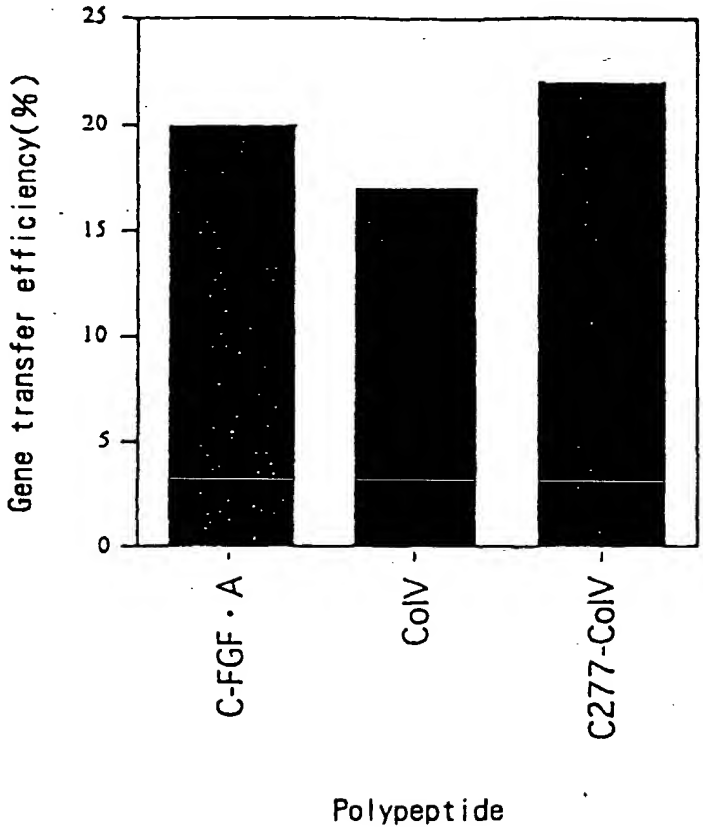

Gene transfer efficiency(%)
25
20
15
10
5
0
C-FGF・A
ColV
C277-ColV
Polypeptide

Fig. 19

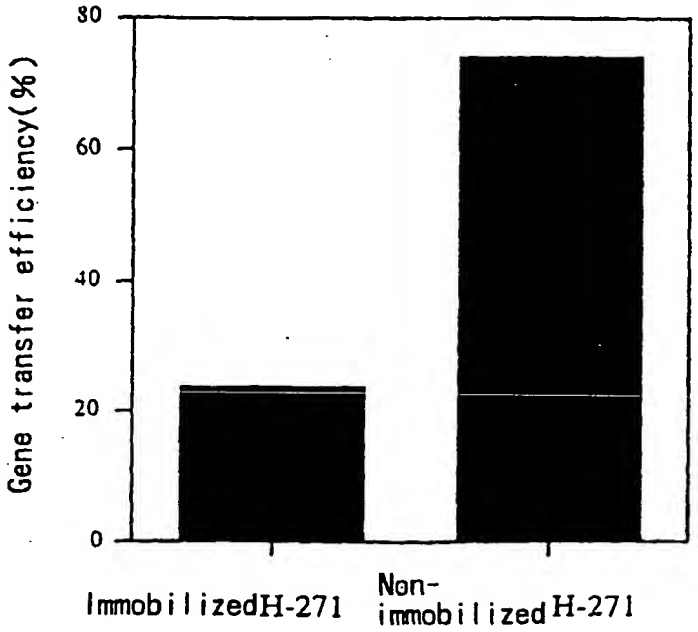

80
60
40
20
0
Gene transfer efficiency(%)
Immobilized H-271
Non-immobilized H-271

Fig. 20

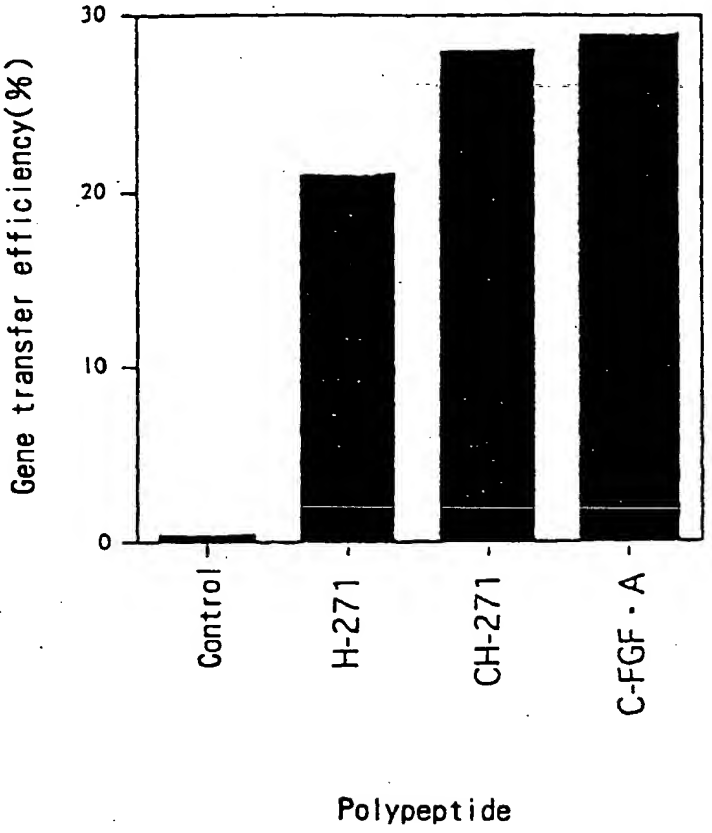

30
20
10
0
Gene transfer efficiency(%)
Control
H-271
CH-271
C-FGF・A
Polypeptide

Fig. 21

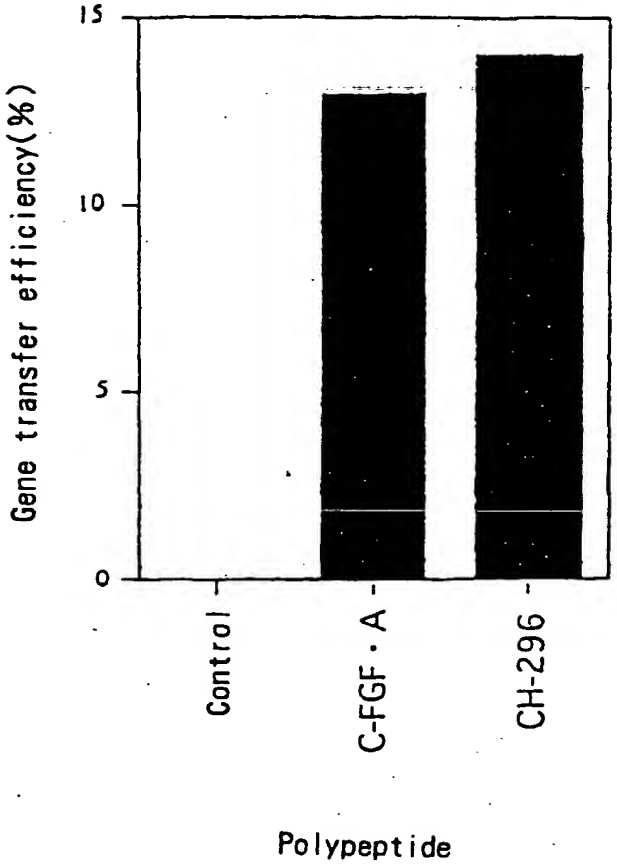

15
10
5
0
Gene transfer efficiency(%)
Control
C-FGF・A
CH-296
Polypeptide

Fig. 22

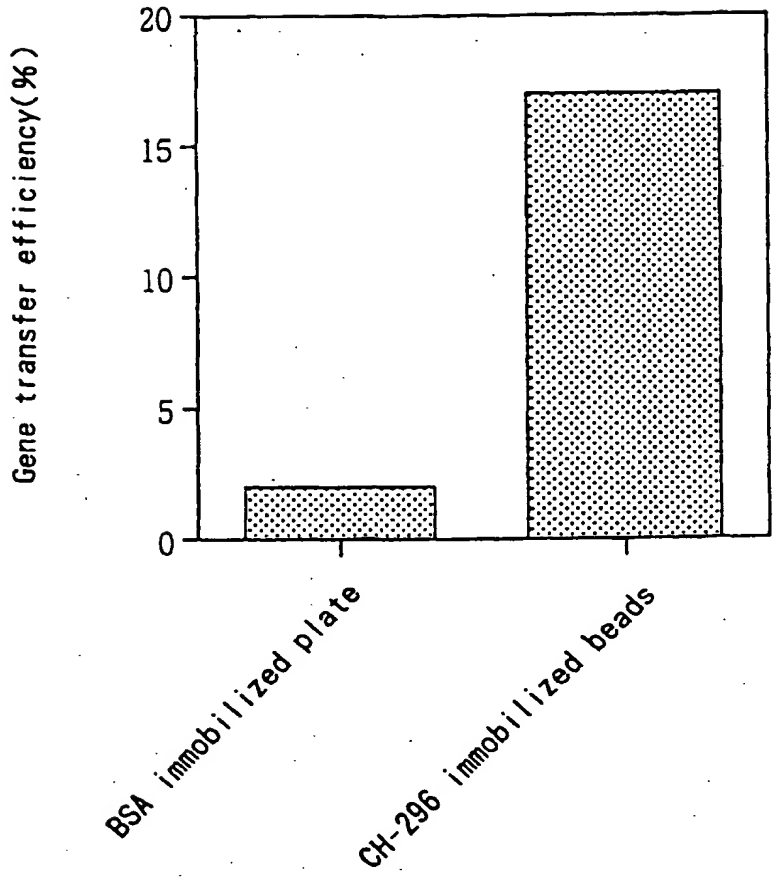

Gene transfer efficiency(%)
20
15
10
5
0
BSA immobilized plate
CH-296 immobilized beads

Fig. 24

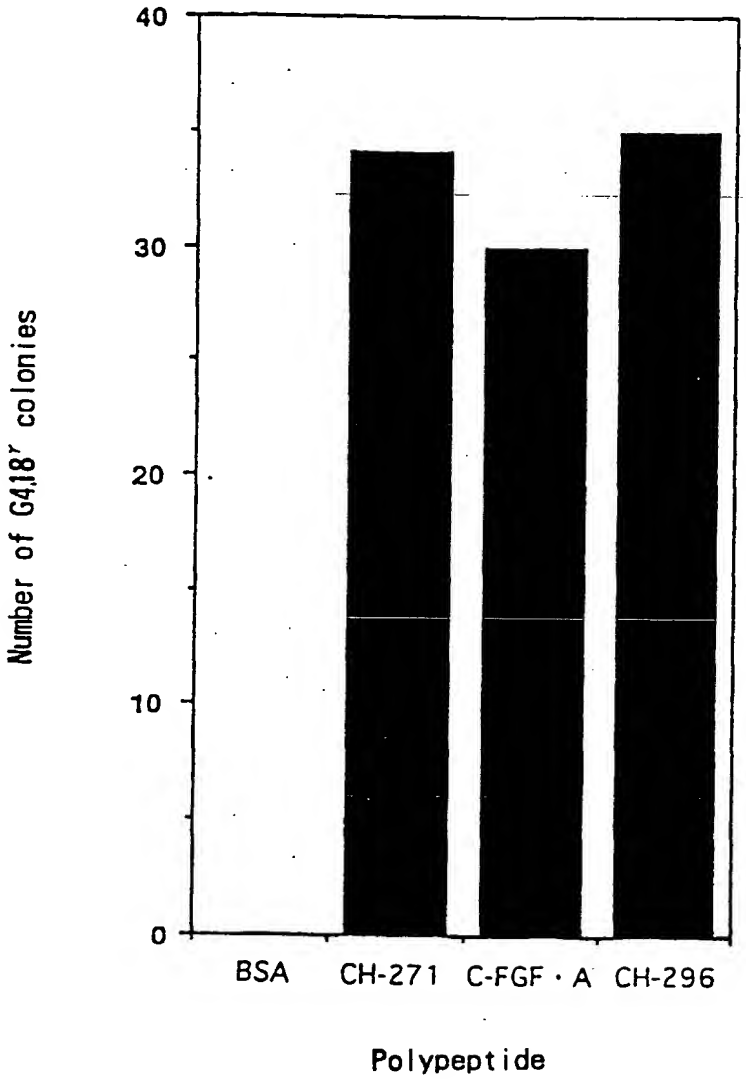

40
30
20
10
0
Number of G418r colonies
BSA
CH-271
C-FGF・A
CH-296
Polypeptide

Fig. 25

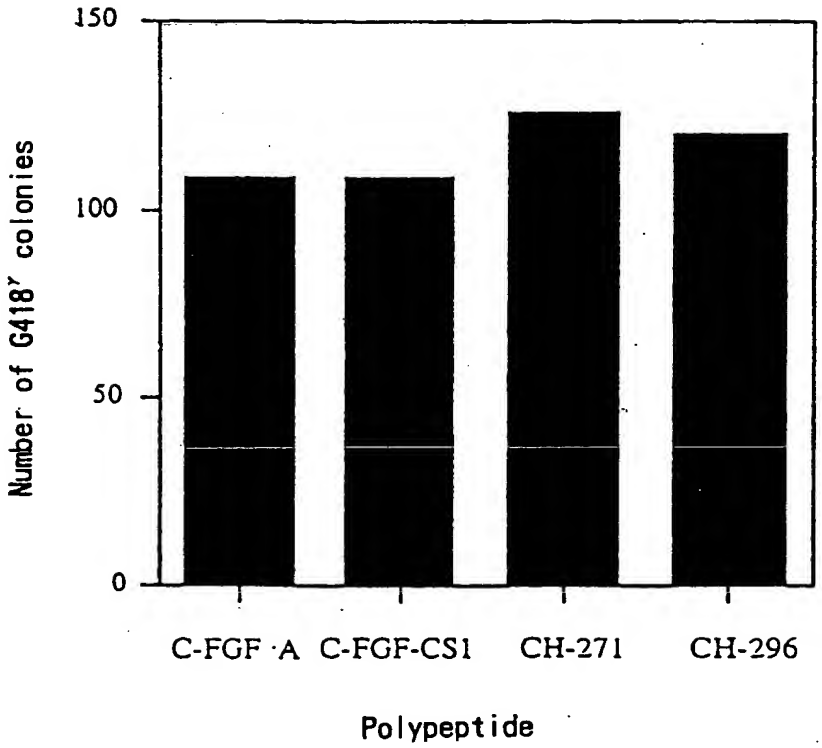
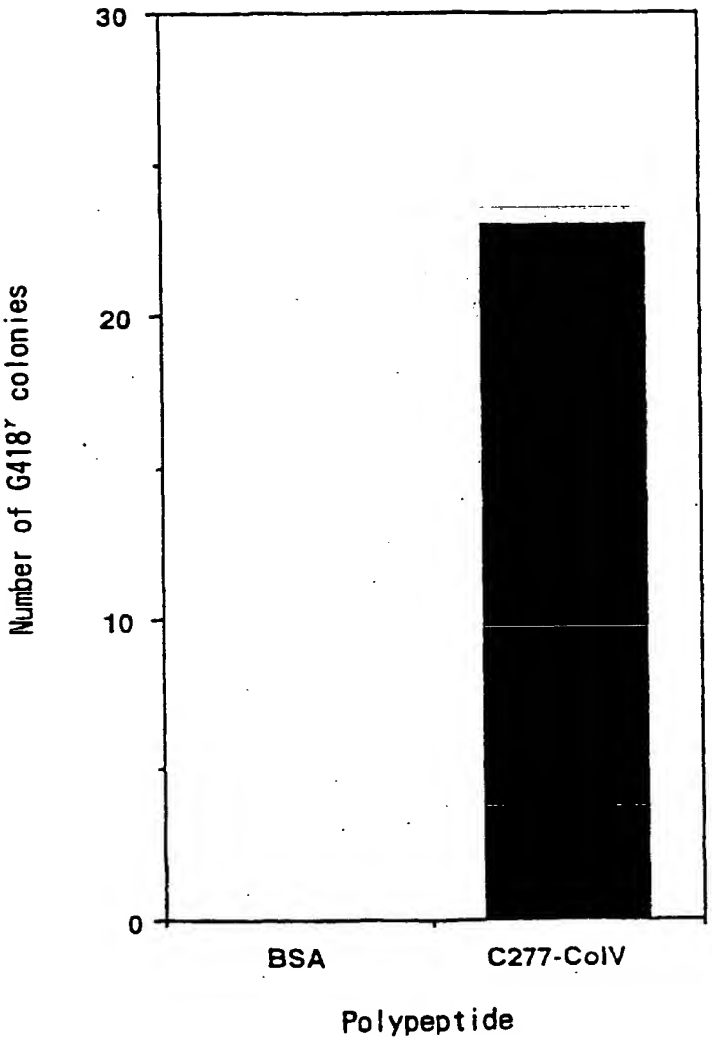

150
100
50
0
Number of G418r colonies
C-FGF-A
C-FGF-CS1
CH-271
CH-296
Polypeptide

Fig. 26


30
20
10
0
Number of G418^{r} colonies
BSA
C277-ColV
Polypeptide

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/03254

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl^6 C12N15/86, C12N15/12, C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int. Cl^6 C12N15/86, C12N15/12, C12N5/10

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPI, BIOSYS, GENETYX-CD

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, 92/17210, A (Boehringer Ingelheim International GMBH), October 15, 1992 (15. 10. 92) & DE, 4110409, A & EP, 577648, A & JP, 6-505980, A (Refer to claims 1, 10, 11, 16, 21, 24; pages 23 to 25)	25-28, 32-37, 43-44, 46-48, 51-52, 54-55, 58-59, 61-62, 65-66, 68-72, 75-80
X A	WO, 95/26200, A (Indiana University Foundation), October 5, 1995 (05. 10. 95) & AU, 9521979, A (Claim; pages 17, 26 to 28)	25-28, 31 1-24, 29-30, 32-89
A	Blood, Vol. 84(10 Suppl. 1) (1994) E.L.W. Kittler et al. "Enhancement of Retroviral Integration by Cytokine Stimulation Impairs Engraftment of Bone Marrow Cells into Non-Myeloablated Hosts" p. 344a (Refer to column 1360)	1 - 32
A	Virology, Vol. 194 (1993) Hugo Soudeyns et al.	1 - 32

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
January 30, 1997 (30. 01. 97)	February 12, 1997 (12. 02. 97)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP96/03254

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	"Identification of a Novel Glucocorticoid Response Element within the genome of the Human Immunodeficiency Virus Type 1" p. 758-768	
A	Blood, Vol. 82(11)(1993) Gay M. Crooks et al. "Growth Factors Increase Amphotropic Retrovirus Binding to Human CD34+ Bone Marrow Progenitor Cells" p. 3290-3297	1 - 32
A	JP, 62-089699, A (Delta Biotechnology Ltd.), April 24, 1987 (24. 04. 87) & EP, 207751, B & DE, 3675591, G	81 - 83, 87 - 89
A	JP, 7-504812, A (Scripps Res Inst), June 1, 1995 (01. 06. 95) & WO, 93/11229, A & EP, 61989, A & US, 5492890, A	84 - 86
A	JP, 63-501953, A (Synergen Inc.), August 4, 1988 (04. 08. 88) & WO, 87/03885, A & EP, 226181, B & US, 5026839, A & US, 4994559, A	87 - 89
A	Nucleic Acids Res., Vol. 16(8)(1988) Paolella G. et al. "Sequence analysis and in vivo expression show that alternative splicing of ED-B and ED-A regions of the human fibronectin gene are independent events" p. 3545-3557	81 - 83

Form PCT/ISA/210 (continuation of second sheet) (July 1992)